THE DISTRIBUTION OF THE MAGNITUDES OF THE INTER-MENSUAL CORRELATION COEFFICIENT FOR EGG PRODUCTION IN THE FIRST TWO LAYING YEARS IN THE DOMESTIC FOWL[1]*

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INTRODUCTION

During the past few years the problem of the correlation between the egg record of the several months of the first laying year, the pullet year, of the domestic fowl has received considerable attention.

As far as I am aware, coefficients were first published for the correlation between the October production and that of the other eleven months of the year in connection with investigations on the relationship between body pigmentation and egg record in the White Leghorn breed (HARRIS, BLAKESLEE and WARNER 1917; HARRIS, BLAKESLEE, WARNER and KIRKPATRICK 1917; BLAKESLEE, HARRIS, WARNER and KIRKPATRICK

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1917). In a second investigation of the problem, the relationship between the production of November, January, April, August and October, and that of all the other months of the year was considered (HARRIS, BLAKESLEE and KIRKPATRICK 1917, 1918). As a result of this investigation, two laws concerning the relationship between the egg production of the months of the first laying year were formulated, as follows:

(1) The correlation between minor periods of time (months) in the year tends to decrease as these periods become more widely separated.

(2) There is more intimate correlation between the autumn and winter months at the beginning and end of the contest year than between the production of these months and the production of the spring and summer months.

To some degree these two laws tend to be mutually obscurant, but their generality for the production of the months of the first year has been demonstrated by more exhaustive studies of a number of independent series. These comprise investigations on the Rhode Island Red breed as studied at the MASSACHUSETTS AGRICULTURAL COLLEGE (HARRIS and GOODALE 1922) and in the INTERNATIONAL EGG LAYING CONTEST at Storrs, Connecticut, and on the White Wyandotte breed (unpublished constants). An analysis of data for the Plymouth Rock breed and further investigations on the White Leghorn breed are in progress. Ultimately, a detailed comparison between these various breeds is to be drawn.

The investigations cited above have had to do with first-year (pullet-year) production only. Constants showing the relationship between all the possible combinations of the individual months of the first and of the second laying year have already been made for the White Leghorn breed as studied at the VINELAND INTERNATIONAL EGG LAYING AND BREEDING CONTEST (HARRIS and LEWIS 1922a). These researches indicate that there are definite laws underlying the distribution of the magnitudes of the correlation coefficients between the minor periods of the first and second year.

The purpose of the present paper is two-fold:

(1) To present the correlation coefficients measuring the relationships between the egg records of all the individual months of the first year and between all the individual months of the second year, and to compare the magnitudes of these coefficients for the two years.

(2) To consider the distribution of the magnitudes of the correlation coefficients measuring the relationship between the twenty-four individual months of the first and second year.

In view of the fact that the productions of the individual months of the first and second years have been shown to be correlated, it seems altogether reasonable to treat the two years as a unit, and to study the distribution of the magnitudes of the correlations throughout the entire period.

This *a priori* assumption is fully justified by the results detailed in the following pages.

The results presented here, considered in connection with the demonstration of correlations between the annual productions of years later than the first two on the basis of the data for the White Leghorn fowl, at the UTAH AGRICULTURAL EXPERIMENT STATION (BALL, TURPIN and ALDER 1914; BALL, ALDER and EGBERT 1916; BALL and ALDER 1917), indicate the desirability of investigations of the correlations between months for periods longer than two years. Such studies would, however, be exceedingly laborious because of the enormous number of permutations of the individual months. The present study has required the determination of 276 correlation coefficients. To carry the study to the third year would require the calculation of 630 coefficients of correlation between the productions of the various months.

MATERIALS AND METHODS

The records upon which the present study is based are those of 443 single-comb White Leghorn birds which completed the first two years of the VINELAND INTERNATIONAL EGG LAYING AND BREEDING CONTEST, held under the supervision of the NEW JERSEY AGRICULTURAL EXPERIMENT STATION.

Details concerning the conduct of this contest have already been given elsewhere (LEWIS 1917, 1918; LEWIS, HANNAS, RALSTON and WENE 1920). Further statistical data are given in four earlier publications on the White Leghorn breed (HARRIS and LEWIS 1921, 1922 a, b, 1923).

The statistical methods employed are those familiar to all serious biometric students.

By inter-mensual correlation we designate the correlation between the production of any two months of the bird's life. By intra-annual correlation as used in the present paper we mean the inter-mensual correlations for the months of the same year. By inter-annual correlation we understand the inter-mensual correlations in which one of the months is in the first laying year and the other in the second laying year.

It will be convenient to designate the whole series of intra-annual correlations for the first or for the second year as a system of correlation coefficients. Thus we may refer to the intra-annual system for the first

year, or to the intra-annual system for the second year. Similarly, we may designate the inter-mensual correlations for the months of the first and of the second year as an inter-annual system for the first and second year.

In the analysis of the three systems of coefficients (which constitute a major system) it becomes necessary to consider the distribution of the magnitudes of the possible correlations between the production of an individual month and those of all other months associated with it as secondary variables. Such a series may be called a minor system of correlations, and may be designated by the month which forms the primary variable in all the combinations.

PRESENTATION AND ANALYSIS OF CONSTANTS

The relationship between the records of the individual months of the year (intra-annual correlation)

It is most convenient to lay the correlations between the records of the individual months of the second year and those between the individual months of the first year side by side in the same table. The constants are arranged symmetrically in table 1. The upper entry of the three which are given for each combination of months is the constant for the second year. The second entry is the constant for the first year. It will be convenient to designate the series of inter-mensual coefficients for one year as a system of correlation coefficients. We have, therefore, to consider the systems for first and second years, respectively.

The inter-mensual, intra-annual correlation coefficients for the first and second year have been abstracted from table 1 and are arranged according to magnitude in table 2. It is clear that the great majority of the constants are low, but generally positive in sign. The average values and the probable errors will be considered below.

As a basis of comparison of the first- and second-year systems, we have determined the differences between the constants for the first and second laying years. These have been taken: constant for second year less constant for first year. Thus, a positive sign indicates a closer degree of correlation in the second than in the first year.

We have to consider: (a) the possible difference in the average value of the inter-mensual correlation in the two years; (b) the distribution of the individual differences, and the comparison of the differences with their probable errors in order to determine whether certain months have higher correlations in the second than in the first year, or *vice versa*; and (c) the consistency of the magnitudes of homologous inter-mensual corre-

TABLE 1

Correlation coefficients measuring the relationship between the egg production of the individual months of the first year and between the individual months of the second year of the bird's life, and differences between these coefficients. Upper entry = second year, second entry = first year, lower entry = difference.

	NOVEMBER x_1	DECEMBER x_2	JANUARY x_3	FEBRUARY x_4	MARCH x_5	APRIL x_6	MAY x_7	JUNE x_8	JULY x_9	AUGUST x_{10}	SEPTEMBER x_{11}	OCTOBER x_{12}
November x_1	.....	+.4463 ± .0257 +.6170 ± .0199 −.1707 ± .0324	+.0906 ± .0318 +.3356 ± .0284 −.2450 ± .0427	+.1272 ± .0315 +.2098 ± .0306 −.0827 ± .0440	+.0785 ± .0319 +.2132 ± .0306 −.1347 ± .0442	−.0085 ± .0320 +.1745 ± .0311 −.1830 ± .0446	−.0274 ± .0320 +.1437 ± .0314 −.1711 ± .0448	−.0324 ± .0320 +.0517 ± .0320 −.0841 ± .0452	+.0019 ± .0320 +.0845 ± .0318 −.0826 ± .0452	+.1161 ± .0316 +.1044 ± .0317 +.0116 ± .0448	+.2524 ± .0300 +.1586 ± .0312 +.0938 ± .0433	+.3396 ± .0284 +.1290 ± .0315 +.2106 ± .0424
December x_2	+.4463 ± .0257 +.6170 ± .0199 −.1707 ± .0324	.....	+.2299 ± .0304 +.6309 ± .0193 −.4010 ± .0360	+.2040 ± .0307 +.2597 ± .0299 −.0557 ± .0429	+.1167 ± .0316 +.1292 ± .0315 −.0125 ± .0446	+.0245 ± .0320 +.1086 ± .0317 −.0851 ± .0450	−.0373 ± .0320 +.1188 ± .0316 −.1561 ± .0450	−.0212 ± .0320 +.0675 ± .0319 −.0888 ± .0452	+.0223 ± .0320 +.1089 ± .0317 −.0866 ± .0450	+.0988 ± .0317 +.2030 ± .0307 −.1042 ± .0442	+.1635 ± .0312 +.2792 ± .0296 −.1157 ± .0430	+.1772 ± .0310 +.2480 ± .0301 −.0708 ± .0432
January x_3	+.0906 ± .0318 +.3356 ± .0284 −.2450 ± .0427	+.2299 ± .0304 +.6309 ± .0193 −.4010 ± .0360	.....	+.3885 ± .0272 +.4201 ± .0264 −.0316 ± .0379	+.2978 ± .0292 +.2238 ± .0304 +.0741 ± .0422	+.0343 ± .0320 +.1622 ± .0312 −.1279 ± .0447	+.0281 ± .0320 +.1304 ± .0315 −.1023 ± .0449	+.0566 ± .0319 +.0398 ± .0320 +.0168 ± .0452	+.1095 ± .0317 +.0929 ± .0318 +.0167 ± .0449	+.1847 ± .0310 +.1604 ± .0312 +.0243 ± .0440	+.2736 ± .0297 +.3046 ± .0291 −.0310 ± .0415	+.1715 ± .0311 +.2852 ± .0274 −.1137 ± .0428
February x_4	+.1272 ± .0315 +.2098 ± .0306 −.0827 ± .0440	+.2040 ± .0307 +.2597 ± .0299 −.0557 ± .0429	+.3885 ± .0272 +.4201 ± .0264 −.0316 ± .0379	.....	+.4587 ± .0253 +.3973 ± .0270 +.0615 ± .0370	+.0947 ± .0318 +.2413 ± .0302 −.1466 ± .0438	+.0012 ± .0320 +.1329 ± .0315 −.1317 ± .0449	+.1527 ± .0313 +.0325 ± .0320 +.1203 ± .0448	+.1326 ± .0315 +.1455 ± .0314 −.0129 ± .0444	+.2033 ± .0307 +.1773 ± .0310 +.0260 ± .0437	+.2602 ± .0299 +.1957 ± .0308 +.0644 ± .0429	+.1557 ± .0313 +.2066 ± .0307 −.0510 ± .0438
March x_5	+.0785 ± .0319 +.2132 ± .0306 −.1347 ± .0442	+.1167 ± .0316 +.1292 ± .0315 −.0125 ± .0446	+.2978 ± .0292 +.2238 ± .0304 +.0741 ± .0422	+.4587 ± .0253 +.3973 ± .0270 +.0615 ± .0370	.....	+.4094 ± .0267 +.6253 ± .0195 −.2158 ± .0331	+.2965 ± .0292 +.3804 ± .0274 −.0839 ± .0401	+.2673 ± .0298 +.2472 ± .0301 +.0201 ± .0423	+.2735 ± .0297 +.2206 ± .0305 +.0529 ± .0425	+.2514 ± .0300 +.1886 ± .0309 +.0628 ± .0431	+.2445 ± .0301 +.1154 ± .0316 +.1291 ± .0437	+.1390 ± .0312 +.1297 ± .0315 +.0293 ± .0444
April x_6	−.0085 ± .0320 +.1745 ± .0311 −.1830 ± .0446	+.0235 ± .0320 +.1086 ± .0317 −.0851 ± .0450	+.0343 ± .0320 +.1622 ± .0312 −.1279 ± .0447	+.0947 ± .0318 +.2413 ± .0302 −.1466 ± .0438	+.4094 ± .0267 +.6253 ± .0195 −.2158 ± .0331	.....	+.5920 ± .0208 +.5765 ± .0214 +.0154 ± .0299	+.4332 ± .0260 +.2762 ± .0296 +.1570 ± .0394	+.3224 ± .0287 +.2136 ± .0306 +.1089 ± .0420	+.2032 ± .0307 +.1901 ± .0309 +.0128 ± .0436	+.1319 ± .0315 +.1509 ± .0313 −.0190 ± .0444	+.0826 ± .0318 +.1144 ± .0316 −.0318 ± .0449
May x_7	−.0274 ± .0320 +.1437 ± .0314 −.1711 ± .0448	−.0373 ± .0320 +.1188 ± .0316 −.1561 ± .0450	+.0281 ± .0320 +.1304 ± .0315 −.1023 ± .0449	+.0012 ± .0320 +.1329 ± .0315 −.1317 ± .0449	+.2965 ± .0292 +.3804 ± .0274 −.0839 ± .0401	+.5920 ± .0208 +.5765 ± .0214 +.0154 ± .0299	.....	+.6463 ± .0187 +.5384 ± .0228 +.1079 ± .0294	+.4443 ± .0257 +.3208 ± .0288 +.1234 ± .0386	+.2757 ± .0296 +.2430 ± .0302 +.0327 ± .0423	+.1797 ± .0310 +.1379 ± .0314 +.0418 ± .0442	+.0723 ± .0319 +.1070 ± .0317 −.0347 ± .0449
June x_8	−.0324 ± .0320 +.0517 ± .0320 −.0841 ± .0452	−.0212 ± .0320 +.0675 ± .0319 −.0888 ± .0452	+.0566 ± .0319 +.0398 ± .0320 +.0168 ± .0452	+.1527 ± .0313 +.0325 ± .0320 +.1203 ± .0448	+.2673 ± .0298 +.2472 ± .0301 +.0201 ± .0423	+.4332 ± .0260 +.2762 ± .0296 +.1570 ± .0394	+.6463 ± .0187 +.5384 ± .0228 +.1079 ± .0294	.....	+.5926 ± .0208 +.5169 ± .0235 +.0758 ± .0314	+.3671 ± .0277 +.3610 ± .0279 +.0060 ± .0393	+.2202 ± .0305 +.1731 ± .0311 +.0471 ± .0435	+.0786 ± .0319 +.0463 ± .0320 +.0323 ± .0451
July x_9	+.0019 ± .0320 +.0845 ± .0318 −.0826 ± .0452	+.0223 ± .0320 +.1089 ± .0317 −.0866 ± .0450	+.1095 ± .0317 +.0929 ± .0318 +.0167 ± .0449	+.1126 ± .0315 +.1455 ± .0314 −.0129 ± .0444	+.2735 ± .0297 +.2206 ± .0305 +.0529 ± .0425	+.3224 ± .0287 +.2136 ± .0306 +.1089 ± .0420	+.4443 ± .0257 +.3208 ± .0288 +.1234 ± .0386	+.5926 ± .0208 +.5169 ± .0235 +.0758 ± .0314	.....	+.5380 ± .0228 +.5764 ± .0214 −.0383 ± .0313	+.3474 ± .0282 +.1757 ± .0311 +.1717 ± .0419	+.1841 ± .0310 +.0348 ± .0320 +.1492 ± .0441
August x_{10}	+.1161 ± .0316 +.1044 ± .0317 +.0116 ± .0448	+.0988 ± .0317 +.2030 ± .0307 −.1042 ± .0442	+.1847 ± .0310 +.1604 ± .0312 +.0243 ± .0440	+.2033 ± .0307 +.1773 ± .0310 +.0260 ± .0437	+.2514 ± .0300 +.1886 ± .0309 +.0628 ± .0431	+.2032 ± .0307 +.1904 ± .0309 +.0128 ± .0436	+.2757 ± .0296 +.2430 ± .0302 +.0327 ± .0423	+.3671 ± .0277 +.3610 ± .0279 +.0060 ± .0393	+.5380 ± .0228 +.5764 ± .0214 −.0383 ± .0313	.....	+.6411 ± .0189 +.4548 ± .0254 +.1862 ± .0317	+.2729 ± .0297 +.2260 ± .0304 +.0469 ± .0425
September x_{11}	+.2524 ± .0300 +.1586 ± .0312 +.0938 ± .0433	+.1635 ± .0312 +.2792 ± .0296 −.1157 ± .0430	+.2736 ± .0297 +.3046 ± .0291 −.0310 ± .0415	+.2602 ± .0299 +.1957 ± .0308 +.0644 ± .0429	+.2445 ± .0301 +.1154 ± .0316 +.1291 ± .0437	+.1319 ± .0315 +.1509 ± .0313 −.0190 ± .0444	+.1797 ± .0310 +.1379 ± .0314 +.0418 ± .0442	+.2202 ± .0305 +.1731 ± .0311 +.0471 ± .0435	+.3474 ± .0282 +.1757 ± .0311 +.1717 ± .0419	+.6411 ± .0189 +.4548 ± .0254 +.1862 ± .0317	.....	+.6178 ± .0198 +.7065 ± .0161 −.0887 ± .0255
October x_{12}	+.3396 ± .0284 +.1290 ± .0315 +.2106 ± .0424	+.1772 ± .0310 +.2480 ± .0301 −.0708 ± .0432	+.1715 ± .0311 +.2852 ± .0294 −.1137 ± .0428	+.1557 ± .0313 +.2066 ± .0307 −.0510 ± .0438	+.1590 ± .0312 +.1297 ± .0315 +.0293 ± .0444	+.0826 ± .0318 +.1144 ± .0316 −.0318 ± .0449	+.0723 ± .0319 +.1070 ± .0317 −.0347 ± .0449	+.0786 ± .0319 +.0463 ± .0320 +.0323 ± .0451	+.1841 ± .0310 +.0348 ± .0320 +.1492 ± .044	+.2729 ± .0297 +.2260 ± .0304 +.0469 ± .0425	+.6178 ± .0198 +.7065 ± .0161 −.0887 ± .0255	.....

TABLE 2

Distribution of magnitudes of inter-mensual correlation coefficients for first and for second laying year.

RANGE OF CORRELATION	FREQUENCY FIRST YEAR	FREQUENCY SECOND YEAR
−.001 to −.050	..	5
+.001 to +.050	4	6
+.051 to +.100	4	8
+.101 to +.150	15	6
+.151 to +.200	11	9
+.201 to +.250	12	6
+.251 to +.300	4	10
+.301 to +.350	3	3
+.351 to +.400	3	2
+.401 to +.450	1	4
+.451 to +.500	1	1
+.501 to +.550	2	1
+.551 to +.600	2	2
+.601 to +.650	3	3
+.651 to +.700	..	..
+.701 to +.750	1	..
Total	66	66

lations in the systems for the first and second years, respectively,—most conveniently measured in terms of the correlation between the constants of the system of coefficients for the first year and second year.

Comparing first the mean values of the inter-mensual correlation in the two years, we have

For second year,	$0.2199 \pm .0146$
For first year,	$0.2390 \pm .0135$
Difference,	$+0.0191 \pm .0093$

The probable error of the difference between the mean correlation for the months of the first and for the months of the second year may be determined by computing the standard deviation of the differences themselves as shown in table 1, or from the frequency distribution of the differences grouped in classes of uniform interval as given in table 3, or from the formula involving the correlation between the two systems of intra-annual correlation coefficients,

$$\sigma^2_{(r_{iiiiii}-r_{iiii})} = \sigma^2 r_{iiiiii} + \sigma^2 r_{iiii} - 2 r_{iiiiii\sigma r_{iiii}} \sigma r_{iiiiii} \sigma r_{iiii}$$

where $r_{iiiiii\sigma r_{iiii}}$ is the coefficient measuring the correlation between the homologous values of the two systems of intra-annual inter-mensual

correlation coefficients, as indicated by the subscripts *i* and *ii* for the monthly egg productions, *e*, of the first and second years. The coefficient required is given below.

Numerically, the average of the correlations for the second year is slightly lower than that of those for the first year, but the difference is less than twice as large as its probable error and cannot be considered definitely significant.

The frequency distributions of the differences, and the ratios of these differences to their individual probable errors are set forth in tables 2 and 3. Table 2 gives the differences in the actual magnitudes of the correlation coefficients. We note that the differences are for the most part relatively small. Forty-one of the differences fall between −.100 and +.100. Twenty-five of the coefficients fall outside this range. Of these, ten are positive, while fifteen are negative.

TABLE 3

Difference in the correlation coefficients for the two years.

DIFFERENCE IN CORRELATION (SECOND YEAR − FIRST YEAR)	FREQUENCY
−.401 to −.450	1
−.351 to −.400	..
−.301 to −.350	..
−.251 to −.300	..
−.201 to −.250	2
−.151 to −.200	4
−.101 to −.150	8
−.051 to −.100	11
−.001 to −.050	8
+.001 to +.050	15
+.051 to +.100	7
+.101 to +.150	6
+.151 to +.200	3
+.201 to +.250	1
Total	66

TABLE 4

Ratios of differences in correlation to probable errors.

RATIO TO PROBABLE ERROR AND SIGN OF DIFFERENCE IN CORRELATION	FREQUENCY
−11.001 to −12.000	1
−10.001 to −11.000	..
− 9.001 to −10.000	..
− 8.001 to − 9.000	..
− 7.001 to − 8.000	..
− 6.001 to − 7.000	1
− 5.001 to − 6.000	2
− 4.001 to − 5.000	1
− 3.001 to − 4.000	5
− 2.001 to − 3.000	7
− 1.001 to − 2.000	10
− 0.001 to − 1.000	7
+ 0.001 to + 1.000	13
+ 1.001 to + 2.000	7
+ 2.001 to + 3.000	5
+ 3.001 to + 4.000	4
+ 4.001 to + 5.000	2
+ 5.001 to + 6.000	1
Total	66

Turning to table 4 which shows the ratios of the differences in the coefficients for the two years, to their probable errors, we note that only

20 of the ratios fall between -1.0 and $+1.0$, whereas 33 should fall between these limits if variations in the differences were due to the probable errors of random sampling. Thus, it appears that certain of the differences are statistically significant with regard to their probable errors. We must, therefore, conclude that while on the average the constants of inter-mensual correlations for the first and second year are of about the same average magnitude, there are combinations of months in which the two constants differ significantly.

An interesting case is that of the conspicuous difference noted at the top of table 3. This is the difference in the coefficient for December and January. The coefficient has the value $+.230$ in the second year, whereas in the first year it has the value $+.631$. The difference is $-.401 \pm .036$. This must be regarded as clearly significant in comparison with its probable error. If we examine other coefficients in this portion of the year we find that similar, although not so marked, differences are found in the correlations between November and December, and November and January.

A probable explanation lies in the fact that different numbers of birds are laying in these months in the two years. Turning back to the tables of an earlier paper (HARRIS and LEWIS 1922, tables 13 to 15) we may calculate the percentage of these birds which did lay in each of the first three months of the contest in first and second years, respectively. The results show that in November of the second year 78.6 percent of the birds did not lay, whereas in November of the first year only 19.4 percent did not lay. In December of the second year, 74.9 percent of the birds did not lay, whereas in December of the first year only 22.3 percent of the birds did not lay. Finally, in January of the second year, 27.1 percent of the birds did not lay, whereas in January of the first year only 20.5 percent of the birds did not lay.

We may now consider the same kind of evidence, but expressed in a different way. The time of beginning of laying in the two years have been tabulated elsewhere (HARRIS and LEWIS 1923, diagrams 1 and 2). We note that in the first year the great bulk of the birds began to lay very early in November. In the second year this was not the case.

It is altogether probable that the smaller proportion of birds which were really expressing their innate capacities in the early months of the second year, results in the low values of the correlations between the earlier months of this year as compared with those found in the first year. It has already been shown (HARRIS, BLAKESLEE and KIRKPATRICK 1918, pp. 49 to 56) that groups of birds which appear identical on the basis of

an alternative system of classification are in reality heterogeneous, and that supplementary criteria may be used in dividing these groups into finer categories.

While the explanation suggested is probably valid for the particular differences for which it is offered, it cannot be applied to some of the other differences which are apparently significant in comparison with their probable errors. Further discussion of individual discrepancies between the results for the first and second years should probably await the analysis and comparative examination of larger series of data.

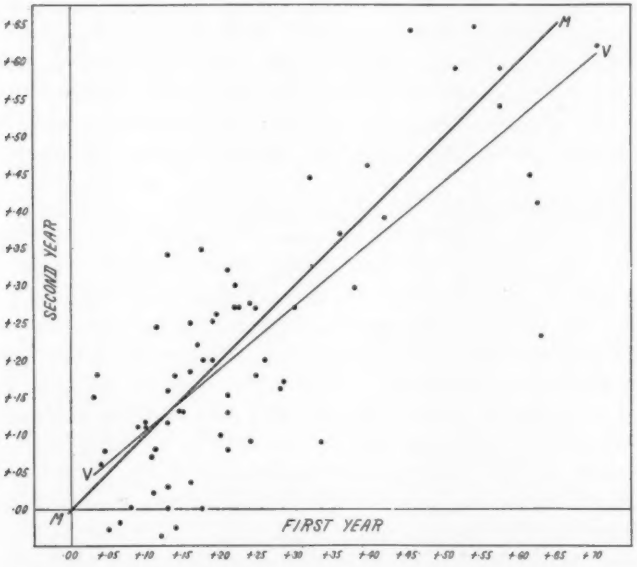

DIAGRAM 1.—Scatter diagram showing the relationship between the values of homologous inter-mensual intra-annual correlations for the first and second years.

The problem of the general consistency of the homologous coefficients of the two years may be most readily solved, on the basis of the data available, up to the present stage of our discussion, and the results of the solution most clearly expressed by the determination of a coefficient of correlation measuring the relationship between the two systems of coefficients. The problem will be taken up from another side later (see page 104).

Working from the entries of table 1 we find a coefficient of correlation between the homologous constants for the first and second year of

$$r_{\text{ieie}\,r_{\text{iiei}}} = .7839 \pm .0320$$

Thus, there is a very high, but by no means perfect, correlation between the systems of constants for the two years.

The closeness of this interrelationship may be shown graphically by representing the positions of the individual pairs of correlations on a plane. On diagram 1 the scale of abscissae represents the magnitudes of the inter-mensual correlations for the first year, while the scale of ordinates represents the magnitudes of the inter-mensual correlations for the second year.

On this diagram the heavy line, M—M, represents equality of correlation for the systems of the first and second year. This line divides the solid dots, which represent the correlation coefficients, into approximately equal groups, thus showing that the difference between the coefficients for the first and the second year is not large.

If the average value and the variability of the values of the coefficients for the two years were the same, a line fitted to the swarm of constants should coincide with the line M—M. The equation to the line is

$$r_{\text{iiei}} = 0.0177 + 0.8459\, r_{\text{iei}}$$

The equation represented by the line V—V is a close approximation to the line M—M.

The relationship between the records of the individual months of the first and second year (inter-annual correlation)

The constants of this system of coefficients have already been published (HARRIS and LEWIS 1922 a, table 4). In subsequent sections they will be considered in their relationship to the intra-annual coefficients and to the distribution of the magnitudes of the coefficients for the whole two-year period.

The relative magnitudes of intra-annual and inter-annual correlation coefficients

We have first to consider the problem of the relative magnitude of the intra-annual and the inter-annual correlation coefficients.

To make the matter quite clear by the use of an illustration, our problem is to determine the relative magnitude of the correlation between the production of November of the first year and December of the second year (the inter-annual correlation) as compared with that between November and December of the first year (the intra-annual correlation)

TABLE 5, PART 1

Comparison between intra-annual inter-mensual correlation coefficients and inter-annual inter-mensual correlation coefficients measuring the relationship between the egg records of the months of the first and second laying years. Under each correlation is given the value of the coefficient divided by its probable error.

Month of first year for inter-annual correlations; month of first or second year for intra-annual correlations

	NOVEMBER e_1	DECEMBER e_2	JANUARY e_3	FEBRUARY e_4	MARCH e_5	APRIL e_6
November e_1	.....	$-.3717 \pm .0409$ 9.08 $-.5423 \pm .0375$ 14.4	$+.0721 \pm .0445$ 1.62 $-.1729 \pm .0422$ 4.09	$+.0782 \pm .0440$ 1.78 $-.0045 \pm .0434$ 0.10	$+.0901 \pm .0445$ 2.02 $-.0446 \pm .0437$ 1.02	$+.0495 \pm .0453$ 1.09 $-.1336 \pm .0446$ 2.99
December e_2	$-.2446 \pm .0401$ 6.11 $-.4153 \pm .0366$ 11.3	.....	$-.0104 \pm .0430$ 0.24 $-.4114 \pm .0361$ 11.4	$+.0675 \pm .0427$ 1.58 $+.0118 \pm .0421$ 0.28	$+.0338 \pm .0445$ 0.76 $+.0212 \pm .0444$ 0.48	$-.0051 \pm .0453$ 0.11 $-.0902 \pm .0453$ 2.00
January e_3	$+.1124 \pm .0442$ 2.54 $-.1325 \pm .0419$ 3.17	$-.0586 \pm .0435$ 1.35 $-.4596 \pm .0366$ 12.6	.....	$-.0910 \pm .0399$ 2.28 $-.1226 \pm .0394$ 3.11	$-.1323 \pm .0428$ 3.10 $-.0582 \pm .0436$ 1.34	$-.0766 \pm .0453$ 1.69 $-.2045 \pm .0447$ 4.58
February e_4	$+.0334 \pm .0444$ 0.75 $-.0493 \pm .0437$ 1.13 0	$-.0036 \pm .0435$ 0.08 $-.0593 \pm .0429$ 1.38	$-.0569 \pm .0394$ 1.44 $-.0885 \pm .0389$ 2.28	.....	$-.2215 \pm .0394$ 5.62 $-.1600 \pm .0405$ 3.95	$-.1296 \pm .0451$ 2.87 $-.2761 \pm .0440$ 6.28
March e_5	$-.0041 \pm .0451$ 0.09 $-.1388 \pm .0442$ 3.14	$-.0951 \pm .0450$ 2.11 $-.1077 \pm .0449$ 2.40	$-.1469 \pm .0428$ 3.43 $-.0729 \pm .0437$ 1.67	$-.2206 \pm .0394$ 5.59 $-.1591 \pm .0405$ 3.93	.....	$-.3156 \pm .0415$ 7.61 $-.5315 \pm .0373$ 14.3
April e_6	$+.0410 \pm .0453$ 0.91 $-.1420 \pm .0446$ 3.18	$-.0525 \pm .0453$ 1.16 $-.1376 \pm .0450$ 3.05	$+.0133 \pm .0452$ 0.29 $-.1146 \pm .0447$ 2.57	$+.0083 \pm .0449$ 0.19 $-.1382 \pm .0438$ 3.16	$-.0919 \pm .0393$ 2.34 $-.3077 \pm .0348$ 8.84	.....

Month of second year for inter-annual correlations; month of first or second year for intra-annual correlations.

TABLE 5, PART 2

Month of first year for inter-annual correlations; month of first or second year for intra-annual correlations		MAY e_7	JUNE e_8	JULY e_9	AUGUST e_{10}	SEPTEMBER e_{11}	OCTOBER e_{12}
	November e_1	$+.1038 \pm .0452$ 2.30 $-.0673 \pm .0447$ 1.50	$+.0856 \pm .0452$ 1.89 $+.0015 \pm .0452$ 0.03	$+.0201 \pm .0453$ 0.44 $-.0625 \pm .0452$ 1.38	$-.0882 \pm .0450$ 1.96 $-.0766 \pm .0451$ 1.70	$-.1269 \pm .0435$ 2.91 $-.0331 \pm .0444$ 0.75	$-.2464 \pm .0426$ 5.79 $-.0358 \pm .0448$ 0.80
	December e_2	$+.0748 \pm .0453$ 1.65 $-.0813 \pm .0450$ 1.81	$+.0907 \pm .0452$ 2.01 $+.0020 \pm .0451$ 0.04	$+.0665 \pm .0451$ 1.47 $-.0200 \pm .0449$ 0.45	$+.0493 \pm .0446$ 1.10 $-.0549 \pm .0439$ 1.25	$+.0977 \pm .0432$ 2.26 $-.0180 \pm .0420$ 0.43	$+.0130 \pm .0438$ 0.30 $-.0578 \pm .0431$ 1.34
	January e_3	$-.0665 \pm .0453$ 1.47 $-.1688 \pm .0449$ 3.76	$-.0282 \pm .0452$ 0.62 $-.0115 \pm .0453$ 0.25	$+.0149 \pm .0447$ 0.33 $+.0316 \pm .0448$ 0.71	$+.0161 \pm .0436$ 0.37 $+.0404 \pm .0438$ 0.92	$+.0472 \pm .0423$ 1.14 $+.0163 \pm .0409$ 0.40	$+.0522 \pm .0435$ 1.20 $-.0615 \pm .0424$ 1.45
	February e_4	$-.0664 \pm .0452$ 1.47 $-.1981 \pm .0448$ 4.42	$-.1279 \pm .0448$ 2.86 $-.0076 \pm .0453$ 0.17	$-.0337 \pm .0447$ 0.75 $-.0466 \pm .0446$ 1.04	$-.0831 \pm .0441$ 1.89 $-.0571 \pm .0443$ 1.29	$-.0478 \pm .0428$ 1.12 $+.0166 \pm .0434$ 0.38	$-.0543 \pm .0445$ 1.22 $-.1052 \pm .0441$ 2.38
	March e_5	$-.2176 \pm .0432$ 5.03 $-.3015 \pm .0420$ 7.17	$-.1777 \pm .0435$ 4.08 $-.1577 \pm .0438$ 3.60	$-.1761 \pm .0434$ 4.05 $-.1232 \pm .0440$ 2.80	$-.1113 \pm .0434$ 2.56 $-.0485 \pm .0441$ 1.10	$-.0834 \pm .0434$ 1.92 $+.0457 \pm .0444$ 1.03	$-.0775 \pm .0446$ 1.74 $-.0482 \pm .0448$ 1.08
	April e_6	$-.4146 \pm .0374$ 11.1 $-.3991 \pm .0377$ 10.6	$-.2772 \pm .0407$ 6.81 $-.1202 \pm .0431$ 2.79	$-.2163 \pm .0428$ 5.06 $-.1074 \pm .0440$ 2.44	$-.0923 \pm .0441$ 2.09 $-.0795 \pm .0442$ 1.80	$-.0156 \pm .0446$ 0.35 $-.0345 \pm .0445$ 0.78	$-.0197 \pm .0451$ 0.44 $-.0514 \pm .0449$ 1.14

Month of second year for inter-annual correlations; month of first or second year for intra-annual correlations

TABLE 5, PART 3

Comparison between intra-annual inter-mensual correlation coefficients and inter-annual inter-mensual correlation coefficients measuring the relationship between the egg records of the months of the first and second laying years. Under each correlation is given the value of the coefficient divided by its probable error.

Month of first year for inter-annual correlations; month of first or second year for intra-annual correlations	NOVEMBER e_1	DECEMBER e_2	JANUARY e_3	FEBRUARY e_4	MARCH e_5	APRIL e_6
May	+.0440 ± .0453	+.0108 ± .0453	+.0058 ± .0453	+.0268 ± .0453	−.1043 ± .0425	−.3110 ± .0361
	0.97	0.24	0.13	0.59	2.45	8.61
e_7	−.1271 ± .0449	−.1453 ± .0450	−.0965 ± .0449	−.1049 ± .0449	−.1882 ± .0413	−.2956 ± .0365
	2.83	3.23	2.15	2.34	4.56	8.11
June	+.0147 ± .0453	+.0378 ± .0453	+.0383 ± .0450	−.1345 ± .0448	−.0830 ± .0429	−.1646 ± .0395
	0.32	0.83	0.85	3.00	1.93	4.16
e_8	−.0694 ± .0453	−.0510 ± .0452	+.0551 ± .0451	−.0143 ± .0453	−.0630 ± .0432	−.0075 ± .0420
	1.53	1.13	1.22	0.32	1.46	0.18
July	−.0462 ± .0453	+.0130 ± .0453	+.0866 ± .0442	−.0685 ± .0448	−.0587 ± .0426	−.1148 ± .0420
	1.02	0.29	1.96	1.53	1.38	2.73
e_9	−.1288 ± .0451	−.0736 ± .0450	+.1033 ± .0443	−.0814 ± .0448	−.0058 ± .0432	−.0059 ± .0433
	2.86	1.64	2.33	1.82	0.14	0.14
August	−.0347 ± .0449	−.0096 ± .0449	+.0161 ± .0436	−.0851 ± .0441	−.0853 ± .0433	−.0625 ± .0439
	0.77	0.21	0.37	1.93	1.97	1.42
e_{10}	−.0230 ± .0449	−.1138 ± .0442	+.0404 ± .0438	−.0591 ± .0443	−.0225 ± .0439	−.0497 ± .0441
	0.51	2.57	0.92	1.33	0.51	1.13
September	−.1846 ± .0438	−.2074 ± .0447	−.1471 ± .0433	−.1288 ± .0434	−.1376 ± .0437	−.1017 ± .0449
	4.21	4.64	3.40	2.97	3.15	2.26
e_{11}	−.0908 ± .0447	−.3231 ± .0435	−.1781 ± .0429	−.0644 ± .0441	−.0085 ± .0448	−.1206 ± .0448
	2.03	7.42	4.15	1.46	0.19	2.69
October	+.0498 ± .0393	−.1580 ± .0446	−.0462 ± .0443	+.0456 ± .0439	−.0650 ± .0446	−.0723 ± .0452
	1.27	3.54	1.04	1.04	1.46	1.60
e_{12}	+.2604 ± .0416	−.2288 ± .0439	−.1599 ± .0432	−.0054 ± .0434	−.0357 ± .0447	−.1041 ± .0450
	6.26	5.21	3.70	0.12	0.80	2.31

Month of second year for inter-annual correlations; month of first or second year for intra-annual correlations

TABLE 5, PART 4

Month of first year for inter-annual correlations; month of first or second year for intra-annual correlations	NOVEMBER c_7	DECEMBER c_8	JANUARY c_9	FEBRUARY c_{10}	MARCH c_{11}	APRIL c_{12}
May c_7	.....	−.3272±.0343 9.54 −.2193±.0367 5.98	−.2126±.0398 5.35 −.0891±.0418 2.13	−.0777±.0427 1.82 −.0450±.0431 1.04	−.0372±.0444 0.84 +.0046±.0444 0.10	+.0073±.0451 0.16 −.0274±.0449 0.61
June c_8	−.2534±.0329 7.70 −.1456±.0354 4.11	.....	−.2870±.0357 8.03 −.2113±.0374 5.65	−.0860±.0405 2.12 −.0800±.0406 1.97	−.0182±.0433 0.42 +.0288±.0437 0.66	−.0237±.0451 0.53 +.0086±.0452 0.19
July c_9	−.0801±.0379 2.11 +.0433±.0400 1.08	−.2057±.0343 6.00 −.1299±.0360 3.61	.....	−.2826±.0376 7.51 −.3210±.0368 8.72	−.1291±.0415 3.11 +.0426±.0436 0.98	−.1168±.0444 2.63 +.0325±.0452 0.72
August c_{10}	−.0087±.0420 0.21 +.0240±.0424 0.57	−.0899±.0406 2.22 −.0838±.0406 2.06	−.1728±.0359 4.81 −.2112±.0351 6.02	.....	−.2756±.0336 8.21 −.0784±.0376 2.37	−.0182±.0422 0.43 +.0286±.0427 0.67
September c_{11}	−.1637±.0446 3.67 −.1219±.0449 2.72	−.1720±.0442 3.89 −.1249∓.0446 2.80	−.0916±.0411 2.23 +0800±.0432 1.85	−.3056±.0341 8.95 −.1194±.0381 3.13	.....	−.2273±.0336 6.76 −.3160±.0315 10.0
October c_{12}	−.1055.±0452 2.34 −.1402.±0450 3.11	−.0730±.0452 1.62 −.0407±.0453 0.90	−.0555±.0442 1.26 +.0938±.0449 2.09	−.0078±.0420 0.19 +.0391±.0426 0.92	.1379.±.0316 4.36 .2266± 0294 7.70	.....

Month of second year for inter-annual correlations; month of first or second year for intra-annual correlations

on the one hand, and November and December of the second year (the intra-annual correlation) on the other. Similar comparisons are to be made for each of 132 of the 144 possible (inter-annual) correlations between the productions of the individual months of the first and second year, and of the homologous combinations within the first and within the second year.

The differences and the probable errors of the differences between the inter-annual and the intra-annual coefficients are given with their probable errors and their ratios to their probable errors in table 5.[2] In this table the upper entry shows the differences between the inter-annual correlations between the individual monthly productions of the first year and those of the second year and the intra-annual correlations of the second year. The lower entry shows the differences between the inter-annual correlations and the intra-annual correlations for the first year. The differences are so taken that a positive sign indicates that the correlation between the productions of the first and second year is higher than that between the individual months of the first or of the second year, as the case may be. A negative sign indicates that the correlation between the months of the different years is lower than that between the months of the same, either first or second.

We may first draw the comparisons on the basis of the mean values for the three series. The constants are:

Inter-annual correlation, first and second year	$+.1439 \pm .0332$
Intra-annual correlation, first year	$+.2390 \pm .0135$
Difference	$-.0951 \pm .0072$
Intra-annual correlation, second year	$+.2199 \pm .0146$
Difference	$-.0760 \pm .0065$

[2] For 12 of the 144 coefficients measuring the relationship between the productions of the months of the first and second year there are no comparable coefficients for the relationship within the same year, since there are only $12 \times 11 = 132$ possible combinations of the months of each year, instead of the $12 \times 12 = 144$ combinations for the two different years.

Table 5 has the same general form as table 1, but because of its large size it has been broken up into four sections as follows:

Part 1	Part 2
First year: November–April	First year: November–April
Second year: November–April	Second year: May–October
Part 3	**Part 4**
First year: May–October	First year: May–October
Second year: November–April	Second year: May–October

The average and standard deviation of the inter-annual correlation coefficient has been calculated on the basis of the 132 coefficients for which comparison with the intra-annual correlations may be made. The probable errors of the differences between inter-annual and intra-annual coefficients have been determined from the standard deviations of the differences as shown in the frequency distributions of table 6.

TABLE 6

Differences between inter-annual and intra-annual correlation coefficients
Differences taken: inter-annual—intra-annual.

DIFFERENCE IN CORRELATION	FREQUENCY FIRST YEAR	FREQUENCY SECOND YEAR
−.501 to −.550	2	..
−.451 to −.500	1	..
−.401 to −.450	2	1
−.351 to −.400	1	1
−.301 to −.350	5	4
−.251 to −.300	2	5
−.201 to −.250	6	10
−.151 to −.200	9	8
−.101 to −.150	26	17
−.051 to −.100	28	30
−.001 to −.050	26	19
+.001 to +.050	19	24
+.051 to +.100	3	11
+.101 to +.150	1	2
+.151 to +.200	..	..
+.201 to +.250	..	..
+.251 to +.300	1	..
Totals	132	132

The differences given above have been calculated from ungrouped values of the coefficients, not from the grouped values as shown in table 6. The values calculated from the grouped constants of the table are, of course, sensibly the same.

The differences are both negative in sign, thus indicating that the inter-annual correlations are somewhat lower than the homologous intra-annual coefficients. The difference between inter-annual and first-year intra-annual is 13.2 times as large as its probable error, and the difference between inter-annual and second-year intra-annual is 11.7 times as large as its probable error. Thus, both differences may be reasonably considered statistically significant.

As a second means of comparison the frequency distribution of the differences may be considered. These are shown in table 6. The frequency

distribution of the ratios of these differences to their probable errors are shown in table 7.

The distribution of the differences in correlation in table 6 shows that by far the greater number of the differences are negative in sign for both of the years.

If we consider that differences ranging from −.050 to +.050 may be regarded essentially zero,[3] we note that for the first-year comparison

TABLE 7

Ratios of differences between inter-annual and intra-annual correlation coefficients to their probable errors.

RATIO TO DIFFERENCE AND SIGN OF DIFFERENCE	FREQUENCY FIRST YEAR	FREQUENCY SECOND YEAR
−14.001 to −15.000	2	..
−13.001 to −14.000	..	..
−12.001 to −13.000	1	..
−11.001 to −12.000	2	1
−10.001 to −11.000	2	..
− 9.001 to −10.000	..	2
− 8.001 to − 9.000	3	4
− 7.001 to − 8.000	3	3
− 6.001 to − 7.000	2	4
− 5.001 to − 6.000	3	6
− 4.001 to − 5.000	6	7
− 3.001 to − 4.000	15	9
− 2.001 to − 3.000	22	18
− 1.001 to − 2.000	28	25
− 0.001 to − 1.000	19	16
+ 0.001 to + 1.000	17	19
+ 1.001 to + 2.000	4	13
+ 2.001 to + 3.000	2	5
+ 3.001 to + 4.000	..	..
+ 4.001 to + 5.000	..	..
+ 5.001 to + 6.000	..	..
+ 6.001 to + 7.000	1	..
Totals	132	132

there are 82 negative differences as compared with 5 which are positive, while for the second year there are 76 negative differences as compared with 13 which are positive in sign.

[3] The probable errors of the differences are of the order 0.040 to 0.045 for the first year and 0.039 to 0.045 for the second year. Thus, the range −.050 to +.050 is approximately coextensive with the probable error for coefficients of this magnitude.

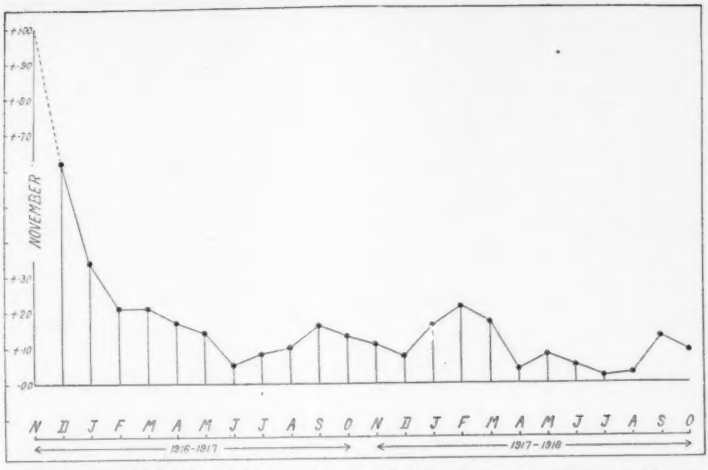

DIAGRAM 2.—Distribution of the magnitudes of the inter-mensual correlation coefficients for November of the first year.

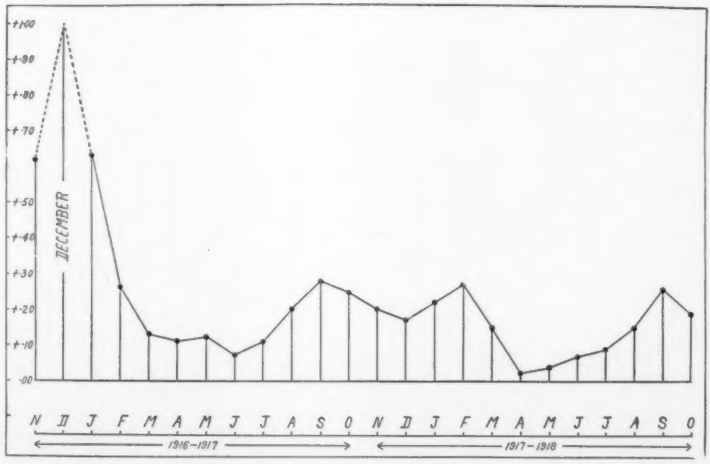

DIAGRAM 3.—Distribution of the magnitudes of the inter-mensual correlation coefficients for December of the first year.

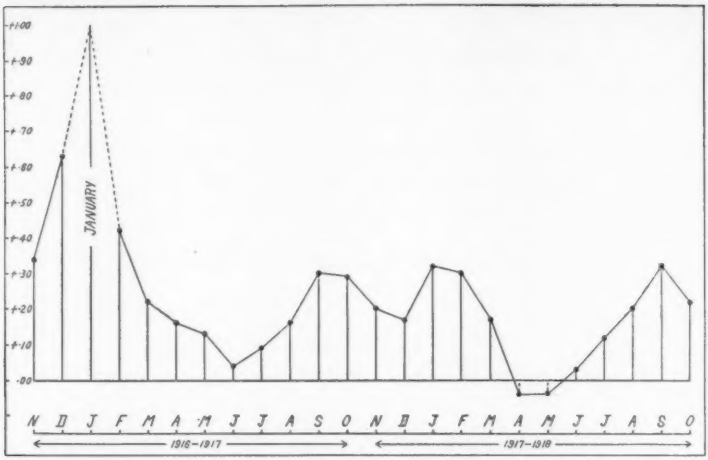

DIAGRAM 4.—Distribution of the magnitudes of the inter-mensual correlation coefficients for January of the first year.

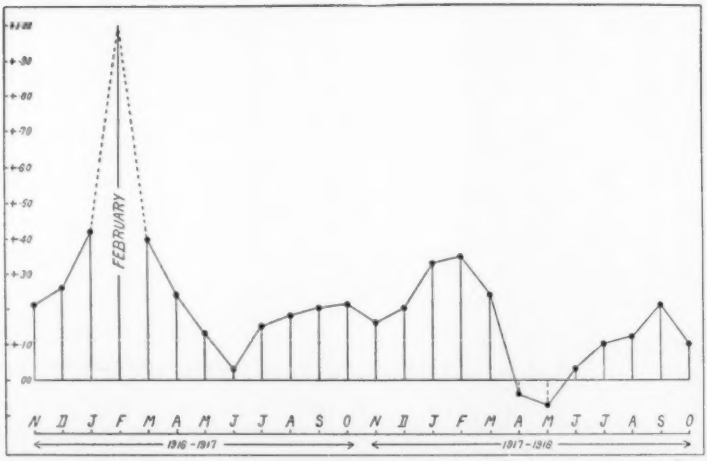

DIAGRAM 5.—Distribution of the magnitudes of the inter-mensual correlation coefficients for February of the first year.

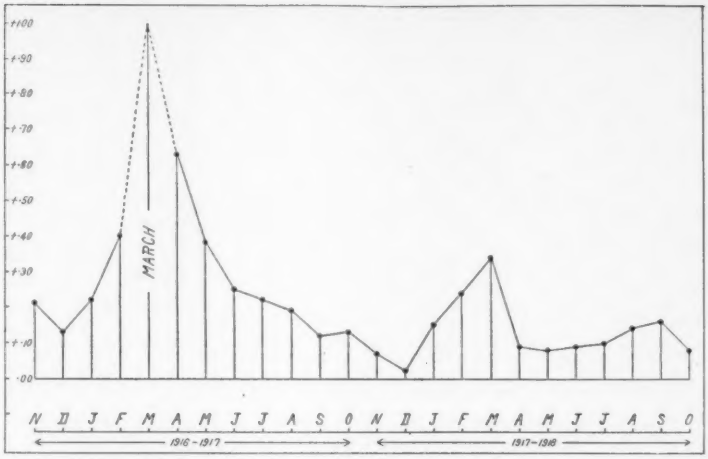

DIAGRAM 6.—Distribution of the magnitudes of the inter-mensual correlation coefficients for March of the first year.

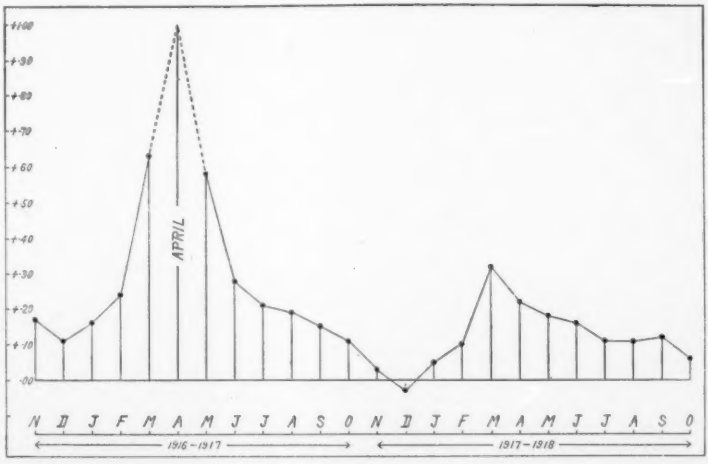

DIAGRAM 7.—Distribution of the magnitudes of the inter-mensual correlation coefficients for April of the first year.

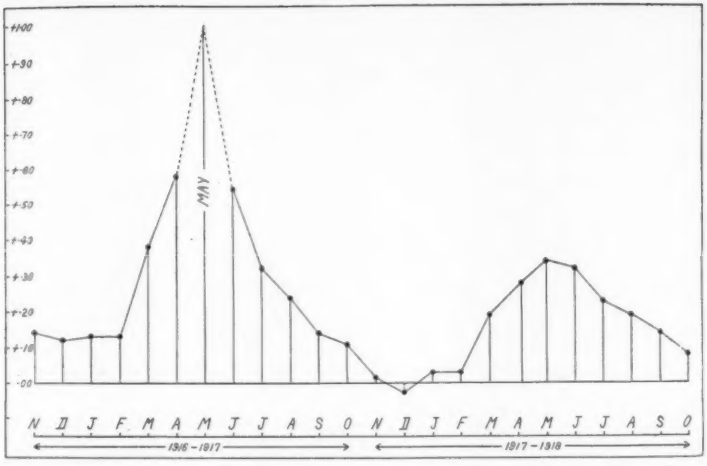

DIAGRAM 8.—Distribution of the magnitudes of the inter-mensual correlation coefficients for May of the first year.

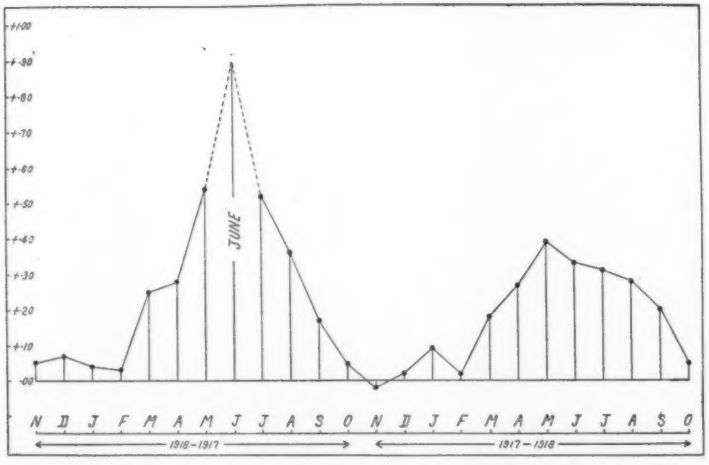

DIAGRAM 9.—Distribution of the magnitudes of the inter-mensual correlation coefficients for June of the first year.

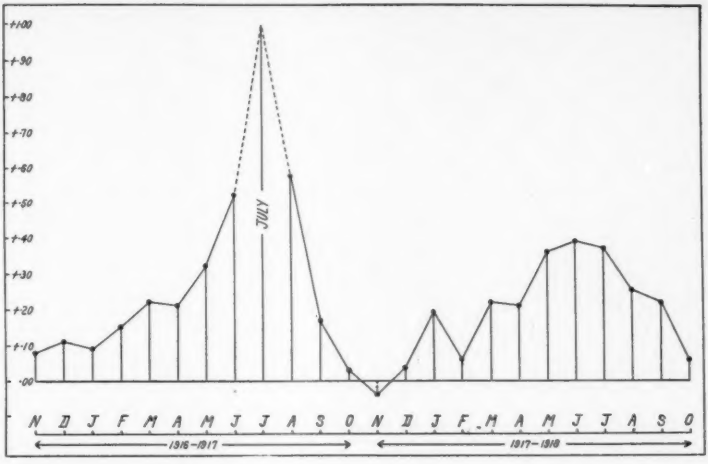

DIAGRAM 10.—Distribution of the magnitudes of the inter-mensual correlation coefficients for July of the first year.

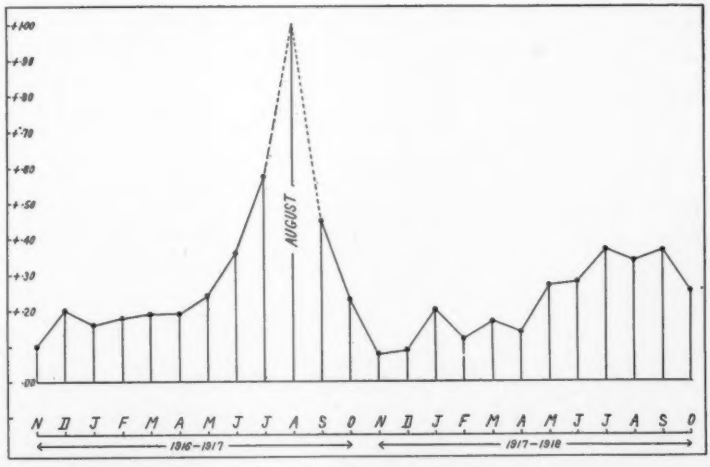

DIAGRAM 11.—Distribution of the magnitudes of the inter-mensual correlation coefficients for August of the first year.

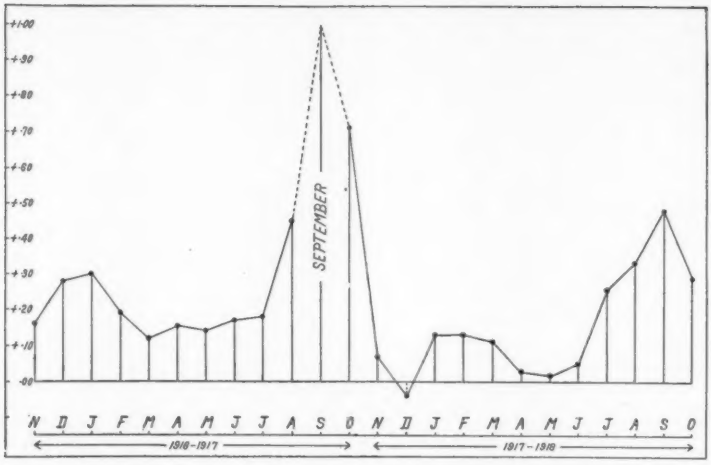

DIAGRAM 12.—Distribution of the magnitudes of the inter-mensual correlation coefficients for September of the first year.

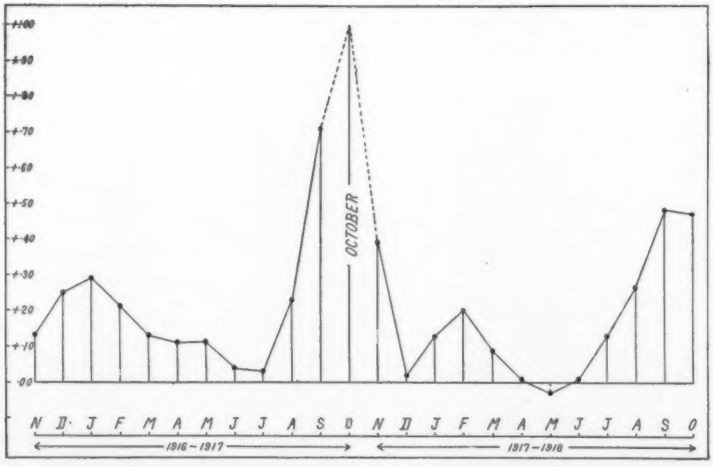

DIAGRAM 13.—Distribution of the magnitudes of the inter-mensual correlation coefficients for October of the first year.

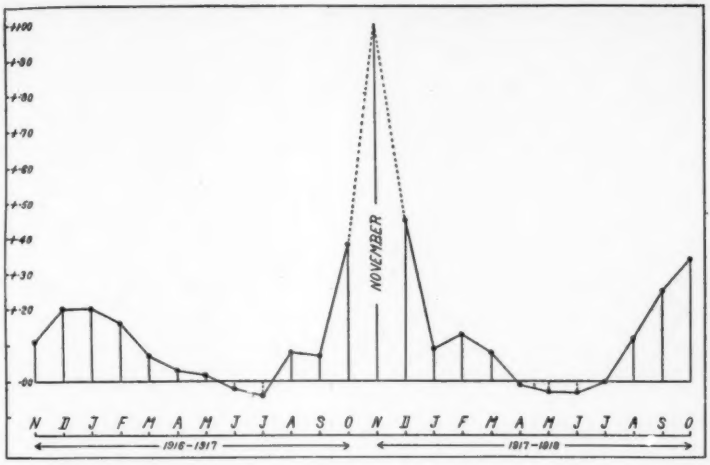

DIAGRAM 14.—Distribution of the magnitudes of the inter-mensual correlation coefficients for November of the second year.

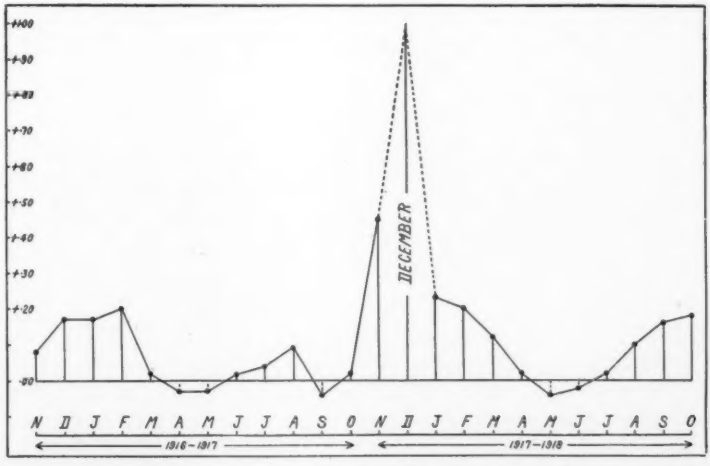

DIAGRAM 15.—Distribution of the magnitudes of the inter-mensual correlation coefficients for December of the second year.

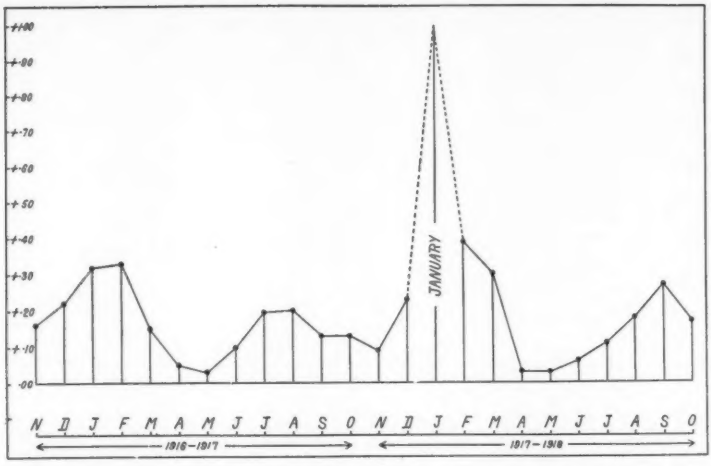

DIAGRAM 16.—Distribution of the magnitudes of the inter-mensual correlation coefficients for January of the second year.

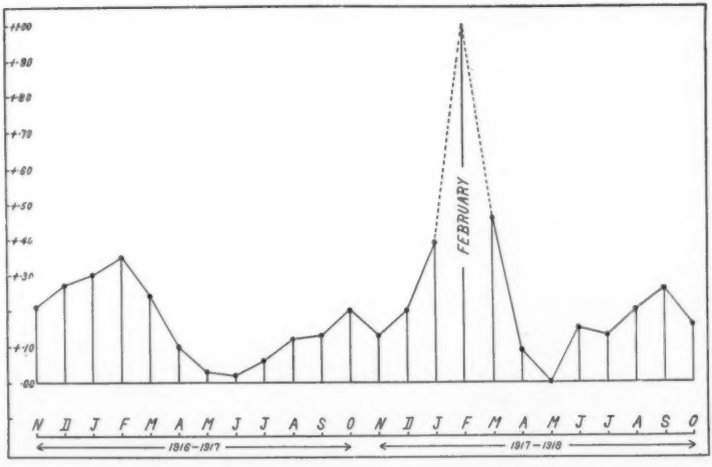

DIAGRAM 17.—Distribution of the magnitudes of the inter-mensual correlation coefficients for February of the second year.

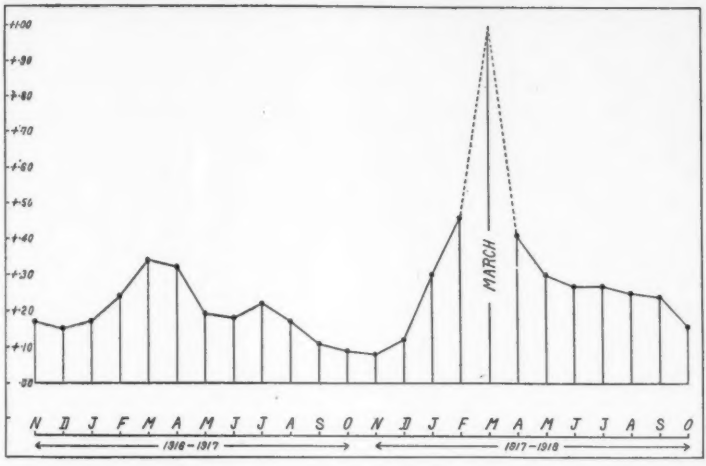

DIAGRAM 18.—Distribution of the magnitudes of the inter-mensual correlation coefficients for March of the second year.

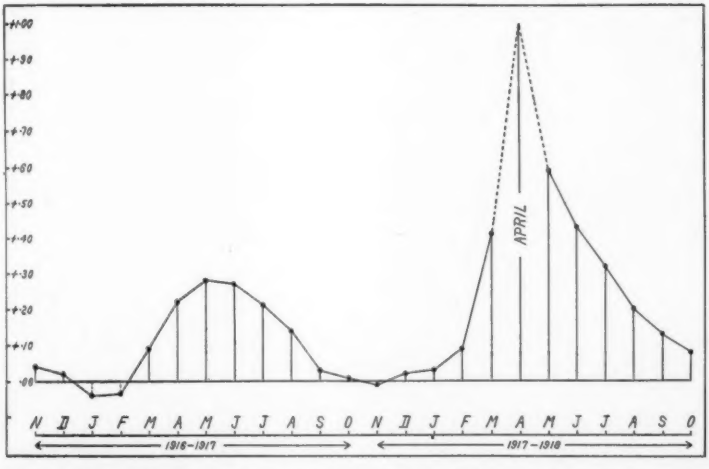

DIAGRAM 19.—Distribution of the magnitudes of the inter-mensual correlation coefficients for April of the second year.

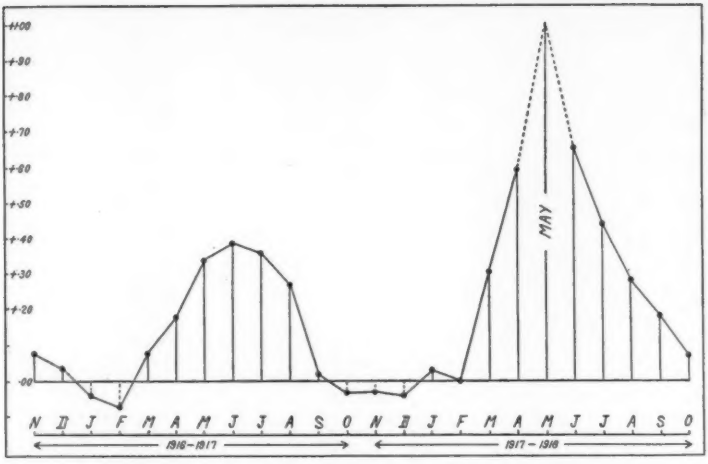

DIAGRAM 20.—Distribution of the magnitudes of the inter-mensual correlation coefficients for May of the second year.

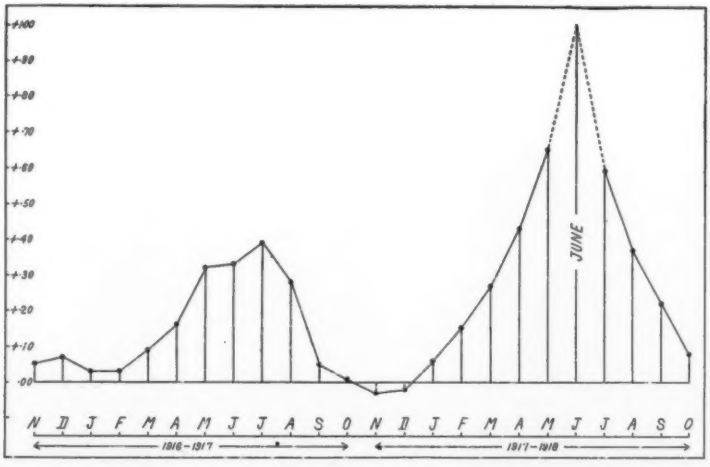

DIAGRAM 21.—Distribution of the magnitudes of the inter-mensual correlation coefficients for June of the second year.

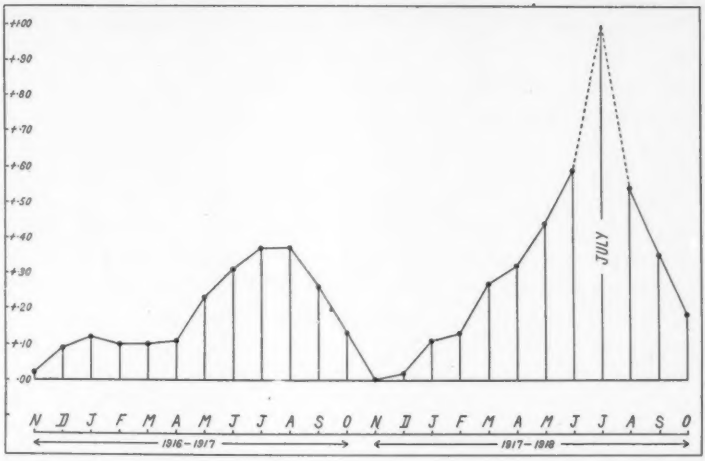

DIAGRAM 22.—Distribution of the magnitudes of the inter-mensual correlation coefficients for July of the second year.

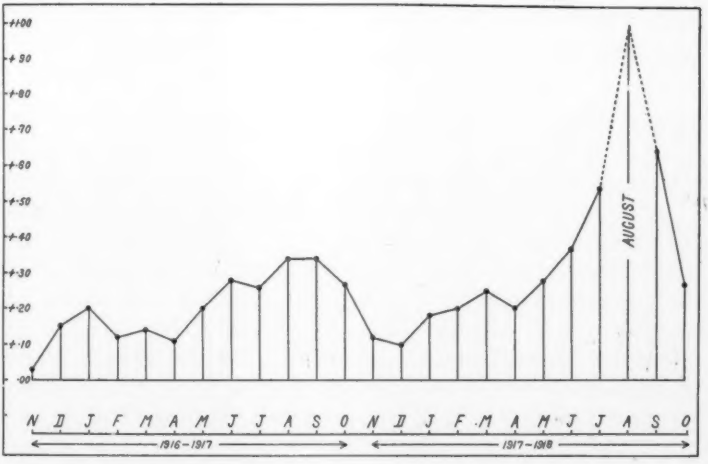

DIAGRAM 23.—Distribution of the magnitudes of the inter-mensual correlation coefficients for August of the second year.

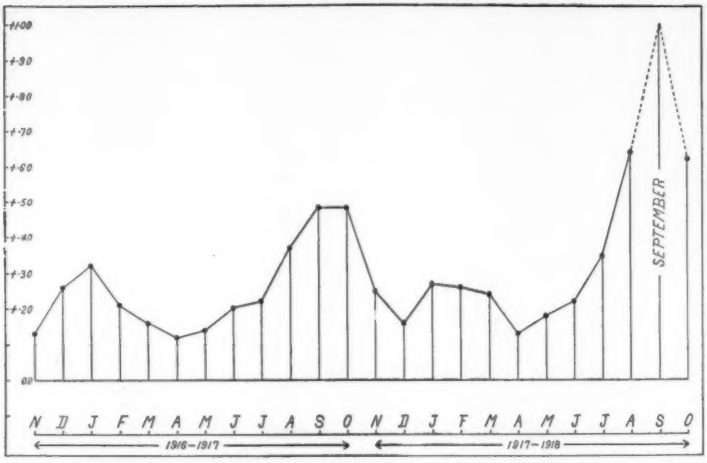

DIAGRAM 24.—Distribution of the magnitudes of the inter-mensual correlation coefficients for September of the second year.

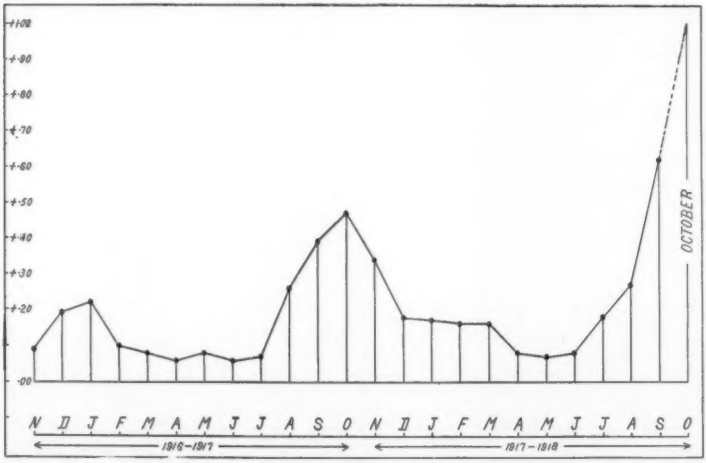

DIAGRAM 25.—Distribution of the magnitudes of the inter-mensual correlation coefficients for October of the second year.

The evidence shows in a wholly conclusive manner, therefore, that in general the intra-annual correlations, for both first and second year, are larger than the inter-annual correlations.

Turn now to the distribution of the ratios of the differences in correlation to their probable errors, as shown in table 7. We may unquestionably consider that differences which fall within the range of their probable errors are not significant. There are 36 such cases for the first year and 35 for the second. These may be disregarded for the purposes of the present discussion. If we consider that differences which are somewhat larger than their probable errors may in the long run be considered significant, we note that there are 89 negative differences as compared with 7 positive differences which are larger than their probable error in the first year. In the second year there are 79 negative as compared with 18 positive differences which may be considered statistically significant. If we regard as statistically significant only differences which are over twice as large as their probable errors, we note that there are 61 significant negative differences in the first year as compared with 3 which are positive. In the second year there are 54 which are negative as compared with 5 which are positive.

These tests of significance of differences fully confirm the conclusions drawn from the examination of the differences themselves, namely, that the intra-annual correlations for both the first and second year are sensibly higher than the inter-annual correlations for the first and second years.

The distribution of the magnitudes of the correlations between the months of the first two laying years

The problem of the distribution of the magnitudes of the correlation coefficients measuring the relationship between the productions of the different combinations of the twenty-four months for which records are available must now be considered.

After the calculation of the 276 correlation coefficients measuring the relationship between all the possible pairs of months in the first two laying years, our problem has been to secure some method of presentation which would bring out clearly the complex interrelationships under consideration.

The method adopted is a graphic one. The 23 coefficients measuring the possible correlations between each month and the 11 other months of the same year and the 12 months of the preceding or following year, as the case may be, are represented on one diagram in the following manner. The 24 months of the two years are distributed serially on the axis of abscissae. The egg productions of these months are regarded as a series

of secondary variables. The correlation between the production of the month which is considered the primary variable with the production of each of these twenty-four months is represented on the scale of ordinates for each of the months. For the purposes of keeping the diagrams for the 24 months an unbroken entirety and of furnishing a convenient standard of comparison, the correlation of the record of an individual month with itself has been properly considered unity. Such perfect correlation coefficients have been connected with the actually determined values showing the relationship between the record of the month under consideration (primary variable) and that of the other months (secondary variable) by broken lines.

Each of the diagrams represents the correlation between one of the 24 months under consideration and each of the other months of the two years. The month used as a basis of classification (primary variable) is indicated by the lettering on the ordinate. Such an arrangement of the correlation coefficients for a month and the other twenty-three months of the two years will for convenience be designated as a system of correlations for the month under consideration.

These diagrams were drawn up from table 4 of a former paper (HARRIS and LEWIS 1922 a) which furnishes the inter-annual coefficients, and from table 1 of the present publication, which gives the intra-annual coefficients for the two years.

Diagrams 2 to 25 give the results for each month, arranged in chronological order from November of the first laying year to October of the second laying year.

A glance over the whole series of diagrams shows that the values of the correlation are practically without exception positive in sign. There are only a few cases in which the coefficients are negative. These are so small numerically that it is difficult, or impossible, to determine whether they actually denote a negative relationship between the months involved, or whether they are merely attributable to the errors of random sampling.

It will be convenient to discuss the systems for the individual months in a practically consecutive order.

The coefficient showing the relationship between the November production of the first year and the production of the subsequent months decreases rapidly from December to June of the first year. It then increases again to attain at least apparent submaxima[4] in September of the

[4] Since the values shown on the charts represent the magnitudes of individual correlation coefficients and not frequencies, it is hardly proper to use the term mode, which is usually employed in the description of frequency distributions, to designate the larger magnitudes. We have, therefore, used the terms maximum and minimum to indicate the highest and the lowest values and submaximum to indicate values which are larger than the general run of constants.

first and February of the second year. The minimum values are on the months of June of the first year and July of the second year.

The results for December of the first year are in general similar to those for November. The empirical minima are on June of the first year and April of the second year. The submaxima are on September of the first year and on February and September of the second year.

The correlation between the production of January of the first year and that of the antecedent and subsequent months shows a decrease in the magnitude of the coefficients measuring the relationship between January production and that of both antecedent and subsequent months as these months become more widely distant in time. Thus the correlation between the January and November production of the first year is materially smaller than that between the January and December production. Similarly, the correlations between January production on the one hand and March, April, May and June production on the other are much smaller than that between January and February production. The minimum values are found on June of the first year and on April and May of the second year. The submaxima are on September or October of the first and on January or February and September of the second year.

The system of correlations for February has a general similarity to that for December. Details may be studied from the diagram. We shall return to a further consideration of the secondary maxima noted in these systems later.

The systems of correlations for March and April of the first year are in general agreement. In both, the minimum value of the coefficient is found on December of the second year. In both, the distribution of magnitudes is bimaximal with the maximum on the months immediately preceding and immediately following the month used as a basis of classification, and with the submaximum on March of the second year.

This result suggests the existence of a hitherto unrecognized law underlying the interrelationship between the productions of the various months of the first two years, namely that *the maximum value of the correlation between widely separated months tends to be that for the combination of homologous months of the first and second years.* Possibly the law may be ultimately made more general by replacing "of the first and second years" by "of successive years."

The suggestion of the existence of this law finds ample confirmation in the results for May, June, July and August of the first year. For each of these four months the distribution of the inter-mensual correlation coefficients is conspicuously bimaximal. The minimum value for the

system occurs on November or December of the second year in each of the four months. The submaximum occurs on either the homologous month of the second year or on a contiguous month. In the case of the system of correlations for May of the first year, the submaximum is on May of the second year. In the case of the system of correlations for June of the first year, the submaximum is on May of the second year, but the correlation for June of the first year and June of the second year is so nearly the same as that for June of the first year and May of the second year that they cannot be asserted to differ significantly. The difference between the two values is only 1.51 times as large as its probable error. In the case of the system of correlations for July, the submaximum is on June of the second year, but the coefficients for July of the first year and May, June and July of the second year are so nearly identical that the slight numerical differences may be due to the errors of random sampling. Finally in the system of correlations for August, the submaximum is on July, August or September of the second year. The value of the correlation for August of the first year and August of the second year is insignificantly smaller than either that for August of the first year and July of the second year or August of the first year and September of the second year.

The systems of correlations for September and October of the first year also confirm the conclusions to be drawn from those for March to August of the first year in so far as the law of the existence of the maximum value of the correlation for widely separated periods on the combination of homologous months is concerned.

The system for September has a conspicuous submaximum on September for the second year. The system for October has a conspicuous submaximum on September or October of the second year; the two coefficients are sensibly identical within the limits of the probable errors of random sampling. The distributions of the coefficients for these two months are sensibly identical.

In these months, however, there appears evidence of the true significance for a wider life cycle of the organism of one of the two laws already recognized on the basis of data for single years only. It has been noted

(HARRIS, BLAKESLEE and KIRKPATRICK 1918, p. 56-69) that

"... there is a more intimate correlation between the autumn and winter months at the beginning and end of the contest year than between the production of these months and the production of the spring and summer months."

The correlations for September and October show a *tendency for the occurrence of secondary maxima on both the earliest months of the first year and the latest months of the second year.*

These results, taken in connection with those for November, December, January and February of the first year, *confirm for the first year* previous conclusions concerning the existence of the law of more intimate correlation between the records of the autumn and winter months at the beginning and end of the contest year than between the records of these months and those of the intervening months. The present result *extends this law to the months of the second year. Previous conclusions are, therefore, fully verified as far as the records of individual years, considered independently, are concerned.*

When we consider the entire systems of correlations for September and October of the first year, instead of limiting attention to the relationships between the production of a single year, the existence of a trimaximal distribution for these months is clearly evident. The system of correlations for November of the second year is also conspicuously trimaximal. That for December is more irregularly so. The results for these two months (November and December of the second year), taken in conjunction with those for September and October of the first year, fully confirm the second law as already stated, and limited, and show that it is applicable to both first- and second-year production.

The two-year distributions of correlations suggest, however, that the greater correlation between the autumn and winter months of the individual years may be merely a phase of a more general law. This possibility must be borne in mind in examining the following systems.

The systems of correlations for January to October of the second year may first be considered with respect to the correlations between these months and subsequent months. The systems for January and February show a submaximum on September of the second year. This submaximum disappears in the systems for March to August of the second year, which, in the correlations with subsequent months, merely exemplify the law of decreasing correlation with increasing separation of the periods of time for which the correlations are determined. It is obviously impossible to test the applicability of this law of decreasing intensity of correlation with increasing separation of the two variables in time as far as subsequent months is concerned on the basis of the system for September of the second year, which has only one subsequent month, or on the basis of that for October of the second year which is the final month of the series.

Turning now to the systems of correlations measuring the relationship between the productions of March to October of the second year and the productions of antecedent months in the second and in the first year, we may note the following facts regarding the distributions.

In all eight months, the correlation with successively earlier months decreases in magnitude for from three to eight months at a varying rate but with some regularity in each case. It then increases to one or more secondary maxima. The distribution is conspicuously bimaximal in the systems for March, April, May, June and July (all of the second year). In the system for March of the second year, the submaximum is on March of the first year. In the system for April of the second year, the first year maximum is on May, but the magnitude for April of the second year and April and June of the first year are sensibly the same as that for April of the second year and May of the first year. In the system for May of the second year the maximum correlation for the first year is that for June, but the coefficients for May of the second year and May, June and July of the first year are practically the same. In the system for June of the second year, the first-year maximum (submaximum of the system) is on July. In the system for July of the second year, the first-year maximum is on July or August, which have coefficients of almost exactly the same magnitude.

The distributions of magnitudes for these five months of the second year confirm in a striking manner, therefore, the results for the occurrence of a submaximum on the homologous month of the second year noted above for the systems for May, June, July and August of the first year.

Bimaximality is not so sharply marked in the systems for August, September, and October of the second year. In the systems for these months there is a rather compelling suggestion of a tertiary maximum which is on January in all the systems. Leaving this tertiary maximum out of account for the moment, we may note that in the system for August of the second year, the maximum for the first year is on the sensibly identical coefficients, August or September. In the system for September of the second year, the maximum for the first year is on the sensibly identical coefficients for September of the second year on the one hand and September or October of the first year on the other. Finally, in the system for October of the second year, the maximum for the preceding year is conspicuously on October.

Turning back to the systems for December, January and February of the first year and examining them in the light of the systems for the final months of the second year, we may see evidences for a tertiary peak on the final months of the second year in these systems. In the systems for December, January and February, it is on September of the following year. It is so clearly marked in these cases that there can be little doubt of the reality of its existence.

Inspection of the systems for November and March of the first year will detect suggestions of tertiary peaks on September of the second year.

SUMMARY DISCUSSION AND CONCLUSIONS

The purpose of this paper, which is one of a series dealing with the various problems of the physiology of egg production in the domestic fowl, is to consider the magnitudes and the distribution of the magnitudes of the correlation coefficients measuring the relationship between the egg productions of the individual months of the first two laying years.

We have designated the correlation between the records of any two months of the bird's life as an inter-mensual correlation coefficient. The inter-mensual coefficients which involve the months of the same laying year, either first or second, have been designated as intra-annual coefficients. Those involving months belonging to different laying years have been designated as inter-annual coefficients.

We have designated the whole series of inter-mensual correlations for the first year or for the second year as a system of correlation coefficients. Thus, we refer to the intra-annual system for the first year, and to the intra-annual system for the second year. Similarly, we designate the whole series of inter-mensual correlation coefficients involving the months of the two different years as an inter-annual system for the first and second year. In the analysis of these systems it has been necessary to consider the distribution of the magnitudes of the coefficients between each individual month of the series (considered a primary variable) and each other month of the series (considered a secondary variable). Such a series of coefficients has been called a minor system, or merely a system when no ambiguity can result from such abbreviation. Such minor systems are designated by the month considered as the primary variable in limiting the minor system.

The complete records for the first two laying years for 443 White Leghorn birds studied at the first INTERNATIONAL EGG LAYING AND BREEDING CONTEST at Vineland, New Jersey, makes possible an investigation of the magnitudes of the $\frac{1}{2}(24\times23)=276$ correlation coefficients measuring the relationship between the months of the first two years of the White Leghorn fowl.

While the intra-annual correlation coefficients for the first year have been published for a number of series of birds, and while the inter-annual coefficients for the Vineland series are available (table 4 of HARRIS and LEWIS 1922b) those for the second year have not, heretofore, been determined.

The results show that the intra-annual coefficients measuring the closeness of interdependence of the productions of the various months of the second year are of about the same average value as those of the first-year records of the same series of birds. The average value of the system of intra-annual (inter-mensual) correlations for the first year is 0.2390, whereas that for the system for the second year is 0.2199. The difference between the two systems of coefficients is $0.0191 \pm .0093$.

The intra-annual correlation coefficients measuring the relationship between the productions of the months of the same year are not merely similar in average magnitude, but the distribution of the magnitudes of the inter-mensual coefficients for each of the individual months of the second year bears a close similarity to the distribution of the homologous coefficients for the months of the first year. This may be shown by comparing the right half of diagram 14 with the left half of diagram 2, the right half of diagram 15 with the left half of diagram 3, and so on to the end of the series, where the right half of diagram 25 is compared with the left half of diagram 13.

The general consistency of the inter-mensual correlation coefficients for the first and second year is measured by a coefficient of correlation between the two systems of $0.7839 \pm .0320$. Thus there is a high but by no means perfect correlation between the two systems. Biological explanations for certain of the discrepancies between the coefficients for individual combinations of months are suggested.

The investigation of the distribution of the magnitudes of the inter-mensual correlation coefficients throughout the entire biennial period under consideration is a logical sequel to earlier researches. It has been shown by NIXON (1912), by BALL and ALDER (1917), and by HARRIS and LEWIS (1922 b), that in the White Leghorn at least, there is a material correlation between the egg records of the first two years. In an earlier paper we have shown that this correlation is so close that on the average the production of each individual month of the second year is correlated with the production of each individual month of the first year. In other papers it has been shown that in the White Leghorn, Rhode Island Red and White Wyandotte breeds the distribution of the inter-mensual correlations for the first year is an orderly one, such that it is possible to express the distributions of magnitude of inter-mensual correlation in two laws.

Since a number of studies on the relationship between the production of the first two years of the bird's life indicate that these records are correlated, while other series of studies show that the inter-mensual coefficients

for the first year are distributed in a lawful manner, it seems quite reasonable to assume that the first two years of the bird's life may be regarded as a unit and to enquire concerning the nature of the distribution of the magnitudes of the inter-mensual correlations for this whole period of time.

The results show that when the correlations for the individual years are considered independently, previous conclusions concerning the distribution of the magnitudes of the correlation coefficient measuring the relationships between the production of individual months are substantiated. When these coefficients are considered for a period of two years, however, it appears that the two laws heretofore stated are merely phases of a more general law, namely, that the correlation between any month considered as a primary variable and subsequent or antecedent months considered as secondary variables, first decreases rapidly as the primary and secondary variables become more widely separated, but again increases as the secondary variable approaches a homologous period of time in another year, after which it again decreases. There is, therefore, a rhythmic or periodic change in the magnitudes of the correlation coefficients as primary and secondary variables become more widely separated in time.

While these conclusions are based upon one series of data only, the consistency of the results evidences for their validity.[5] While the present results pertain to the White Leghorn breed only, the fact that other breeds have been found in other studies to obey the two laws originally formulated for the distribution of the correlation coefficients of a single year, justifies the assumption that the law here stated may be found to be applicable to them also.

The explanation of this phenomenon requires more intensive biological and statistical investigation. Its definiteness indicates clearly the importance of such research.

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[5] It is proper to state that analysis of another series of data for the White Leghorn breed does not give results so consistent as those published here, but evidences of untrustworthiness in the second series of data which became known to us only after the analysis was undertaken leads us to feel that they should be discarded.

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MEIOSIS IN THE POLLEN MOTHER CELLS OF *OENOTHERA BIENNIS* AND *OENOTHERA BIENNIS SULFUREA*

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INTRODUCTION

The material upon which this study is based was obtained during the summer of 1920 in the experimental garden of Professor B. M. DAVIS, at Ann Arbor, Michigan. The culture of *Oe. biennis* L. from which fixations were made was the ninth generation in a selfed line descended from seeds obtained originally from Professor HUGO DE VRIES, the strain being called by Professor DAVIS, *biennis* H. It is not to be confused with the *Oe. biennis* studied cytologically by Professor DAVIS, which is a different species derived from American sources (DAVIS 1910). Material of *Oe. biennis sulfurea* was taken from a culture which represented the fourth generation in a selfed line from seed furnished by Professor DE VRIES.

Six extensive fixations of *Oe. biennis* were made from two plants; and five fixations of *Oe. biennis sulfurea* were secured from five individuals. The critical periods of "diakinesis" and the heterotypic metaphase, as

well as all other stages, were plentifully obtained from both plants of *biennis*. I missed both of these important phases, however, in all but one of the fixations of *biennis sulfurea*; but this one fixation has yielded material clearly indicating that in cytological behavior this variety is in all respects similar to *Oe. biennis*.[1]

As fixing solutions, weak Flemming with maltose and urea added, and one of ALLEN'S modifications of Bouin's solution were used. The former failed in large measure, probably because of the waxy layer which coats the anthers, but the fixations obtained with the latter were in general satisfactory, and often excellent. For the formula of this fluid see CLELAND 1924.

DESCRIPTION

Early prophase stages

I do not propose to devote much space to a discussion of the earlier stages of meiosis in these forms, inasmuch as they appear not to differ from corresponding stages in species already described (CLELAND 1922, 1924). I have made a careful study of cells in the archesporial stage and in early prophase, especially in *Oe. biennis*. In general, as well as in particulars, the history conforms closely to that found in *Oe. franciscana* and *Oe. franciscana sulfurea*, and can be summarized briefly as follows:

1. Archesporial cells in the resting stage prior to the beginning of the heterotypic prophase, are characterized (a) by a copious network of delicate threads, which show very little indication of parallel arrangements; and (b) by the presence, usually, of one large nucleolus and several distinctly smaller ones.

2. The process which takes place during early prophase stages may be described as a gradual transformation of the fine-meshed network of delicate threads into a large-meshed system of much coarser threads. This is apparently accompanied by the contraction of certain threads, and their absorption into the body of others, a process that gives rise to unequal stresses and strains within the system, which may help to cause its collapse toward one side of the nucleus, resulting in the stage of synizesis.

[1] In an interesting paper by STERLING H. EMERSON (1924), that has just come to hand, figures are given of the "diakinesis" stage in *Oe. biennis* and *Oe. biennis sulfurea*, clearly showing in both plants the presence of circles, such as are to be described in this paper. Thus, the evidence for the existence of circles composed of chromosomes arranged end to end rests not alone on the few plants examined by me, but upon additional material collected from the same strains, but at a later date. In regard to chromosome behavior during the heterotypic metaphase, however, EMERSON takes exception to the results described in this paper.

3. It is inevitable that such a procedure should result in occasional, even frequent, cases of parallelism of threads, but these are no more numerous or extensive than would be expected in view of the process, and can hardly be considered as evidence of a synaptic conjugation of homologous elements.

4. In these forms, as in those previously studied, the synizetic knot generally lies next to the side of the nucleus which was in all probability first reached by the fixing fluid. This, to my mind, casts suspicion upon the nature and cause of synizesis, and suggests that the phenomenon may be an artifact. The various contractions and adjustments going on in the reticulum at this time may cause it to become torn away from the nuclear membrane, and thus render it easy for even the best fixing fluid to change its position, or even to accentuate the amount of shrinkage. It is no doubt more difficult to fix without shrinkage a thread whose ends are free, than one whose ends are attached to some more or less stable object.

5. The nucleoli are in intimate contact with the reticulum during the resting stage and the heterotypic prophase, and it seems fairly certain that during synizesis material is drawn from these bodies and added to the substance of the network. This is indicated first by the fact that the endonucleolus does not appear until late synizesis, being apparently masked at earlier stages. It is further suggested by the fact that the threads which connect the nucleolus with the reticulum are as a rule much swollen during late synizesis, as though gorged with the outpoured material. Conditions such as are depicted in the paper on *franciscana sulfurea* (CLELAND 1924, figure 3) are occasionally seen, though such striking figures are not common.

6. It is an interesting fact that during the stages when the endonucleolus is apt to appear for the first time, this little body may frequently be observed to have an organic connection with a certain portion of the reticulum (plate 1, figure 1. See also CLELAND 1922, figures 14, 16; 1924, figure 4). I am not at present prepared, however, to suggest what may be the significance of this fact.

The open spireme

Many cases have been seen in which the reticulum, after emerging from synizesis, has become quite evenly distributed throughout the nucleus, so that the individual parts may be easily examined (plate 1, figure 1). In comparison with the total number of cells studied, however, those presenting this picture clearly are quite rare, and it is either a stage very

Explanation of Plates

All figures were drawn with the aid of a camera lucida, using a Spencer compound binocular microscope, and a Bausch and Lomb 2mm apochromatic objective with 25x compensating ocular. They have been reduced one-fourth in reproduction; present magnification approximately 2800 diameters.

All figures represent *Oe. biennis*, except figures 12 and 13, which were drawn from *Oe. biennis sulfurea*.

Plate 1

Figure 1.—The open spireme stage. Threads are single. The endonucleolus is in contact with the reticulum.

Figure 2.—Very early second contraction, the threads in the center becoming greatly swollen, those at the periphery being thrown into radiating loops. Note the close approximation of the sides of some of the loops.

Figures 3, 4, 5.—More advanced stages in second contraction.

Figure 6.—Late second contraction, perhaps beginning to unfold. Individual chromosomes are becoming recognizable.

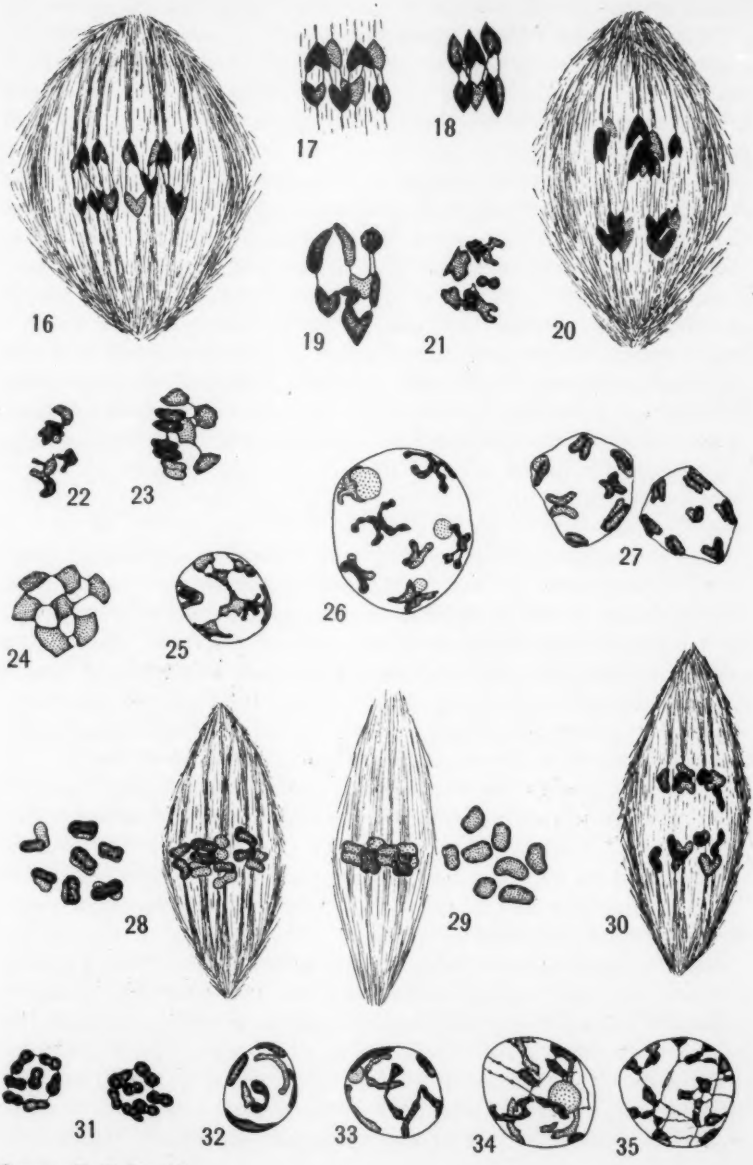
Figure 7.—The unfolding process nearly completed, many of the individual chromosomes clearly visible.

Figures 8, 9, 10.—"Diakinesis," showing the circle of 8 and the circle of 6 linked together.

Figure 11.—The circles have fused to form a circle of 14 chromosomes. The only undoubted case of this observed.

Figures 12, 13.—"Diakinesis" in *Oe. biennis sulfurea*, showing the circle of 8 and the circle of 6. In figure 12 they are linked, in figure 13 they have separated.

Figures 14, 15.—The multipolar-spindle stage with the circles intact. The spindle fibers have been omitted.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quickly passed through, or else in many cells the threads fail to become widely spread, and remain instead in a more or less dense tangle in one part of the nucleus. During this period, one may look in vain for indications of a split in the threads, or of close approximation. The open spireme stage affords very little evidence, therefore, for the presence of parasynapsis.

The threads constituting the open spireme are fairly uniform in diameter throughout the nucleus, and at times appear quite smooth, while at other times there is a more or less marked suggestion of chromomeres. Free ends are rarely found, and are probably normally not present. The system at this time is still in the form of a reticulum, but the meshes are few in number, and quite extensive in size. The large nucleolus continues, as a rule, to maintain a lateral position, but loses its flattened appearance, and once more approximates the original spherical shape. The endonucleolus remains in contact with a part of the spireme for a varying length of time, the connection being broken in some cells before the open spireme stage is formed, and in others not until later.

Second contraction

After a time, the threads begin to lose their evenness of diameter. Those in one portion of the nucleus, usually toward the center, or occasionally near the largest nucleolus, begin to thicken, apparently at the expense of the ones nearer the periphery (plate 1, figures 2 and 3). It becomes evident with the continuance of this process that the peripheral threads are arranged into loops which radiate out in all directions. As second contraction proceeds, the threads in the central portion become thicker and more massed, while the loops gradually contract and are little by little drawn in toward the ever enlarging central region (figures 3 and 4). During this process of contraction, the two sides of individual loops often become closely applied. They are so closely appressed in some cases as almost to give the impression of a single thread, double in thickness (figure 5). All transitions may be noted, however, between this extreme and cases where the loops are very broad.

The loops, gradually shortening, and often losing much of their stainable material, are usually visible until a very late stage,—in fact, it may be rather confidentially asserted that no stage in second contraction has failed to show them. While in a good many cells, at the time of maximum contraction, they have become almost, if not entirely, withdrawn into the central mass, there are nevertheless many other cells which seem to suggest that they need not necessarily entirely disappear. There is in

fact no sure way of telling whether in certain cells the contraction is still going on, whether it is still at its maximum, or whether the cell is beginning to emerge from this condition. We apparently find radiating loops in all stages. They are comparatively thin in diameter in early contraction stages, become gradually thicker during the time of maximum contraction, and when they emerge are noticeably thicker than they were before. There is little doubt in my mind that the loops which emerge from the second contraction stage, as illustrated in plate 1, figures 6 and 7, are the same as those which entered it in the first place (figures 3 and 4). This is evidenced not only by the presence of loops in all stages of second contraction, but also by the fact that during this period one may easily find incipient stages in the differentiation of the individual chromosomes which are soon to make their appearance. Notice, for instance, the loop in the upper left-hand corner in figure 4, and the evident suggestion of individual chromosomes in figures 5 and 6. Further negative evidence is afforded by the lack of indications of a transverse separation at the ends of the loops which enter the contraction stage, and the subsequent longitudinal splitting of the separated halves of these to form the loops which finally emerge. Altogether, I feel that it is almost certain that the spiremes which finally appear in "diakinesis," and which are unquestionably univalent in character, can be traced backward through the second contraction stage by means of the radiating loops, and can be shown to be the same as the open spireme, much shortened and thickened and simplified, but nevertheless the same. The open spireme is without doubt univalent in character, and not bivalent.

One further word should be added with regard to this stage. At or near the time of maximum contraction, one sometimes notes that the sides of the radiating loops appear to be twisted around each other (figure 5). This approach to a strepsinema condition might result occasionally in the kind of chromatin exchange known as crossing over.

"Diakinesis"

When the second contraction knot begins to unfold, it soon becomes evident that the elements which are emerging are not in the nature of paired univalents, as they are in most plants. Instead, we find that in both *biennis* and *biennis sulfurea* there are two spiremes in each nucleus and that each spireme is in the form of a closed circle. One circle is composed of 6 univalent chromosomes attached end to end; the other o 8 chromosomes; and the two circles are usually found linked together (figures 8 to 10, 12, 13). Naturally enough, the linked circles are often

in physical contact with each other at one or more places, and it sometimes happens that contact occurs in such a way that a thread connecting two chromosomes in one circle lies against the connection between two chromosomes of the other circle. In such cases, the circles appear to be fused into a figure 8. An actual fusion of the two threads probably takes place occasionally, for I found one nucleus in *Oe. biennis* in which they had apparently fused and then separated at right angles to the plane in which they had come together, the result being the formation of a large circle of 14 chromosomes (figure 11). Excepting this one case, and the occasional instances in which the linked chromosomes simulate a figure 8, however, the arrangement in the stage just after second contraction is constant, and is one in which two circles, one composed of 6 and the other of 8 chromosomes, are closely linked together. There is therefore no pairing whatever between any of the homologous chromosomes of either plant during this stage, in marked contrast to what we normally expect in diakinesis in most plants.

During the period that follows, leading up to the heterotypic metaphase, certain changes occur in the nucleus. In the first place, the circles of chromosomes become, as a rule, unlinked. We also find that they sometimes break open. In many cases where I have observed open chains instead of circles, the break has been due to the section knife, but numerous instances have been seen in which this appearance could not have been due to any treatment accorded the material. The circles probably break open naturally at times, particularly when they are becoming unlinked. Their separation no doubt involves a temporary break in one of the circles. The broken ends fail to become reunited, and the spireme from that time on appears as a chain of 6 or 8 chromosomes, instead of a closed circle.

A description of such an unusual type of "diakinesis" should be backed by a considerable mass of evidence. I have records of 113 nuclei of *biennis* in which, at this stage, one or both of the spiremes were found to be complete. In 54 cases, both circles were closed and intact; in 21, the circle of 6 was intact, but the other circle was only partially present in the section, or complete but open, in the form of a chain; in 22 cases, the circle of 8 was intact, the circle of 6 either open or incomplete; in 7, the circle of 6 was complete and open, the circle of 8 incomplete or absent; and in 9, the circle of 8 was complete and open, the circle of 6 incomplete or absent. Corresponding figures for *biennis sulfurea* follow: In 14 cases, both circles were closed and intact; in 12 cases, both circles were complete, but one was open; in 3 cases, both were complete and both were open; in

10 cases, the circle of 6 was intact, the rest incomplete or obscure; in 5 cases, the circle of 8 was intact, the rest incomplete or obscure. In both forms, every nucleus that was sufficiently complete and clear to show any arrangement displayed the one just described. There can be no doubt, therefore, I believe, that the condition here described is constant for the species.

During the period of "diakinesis," we find that the chromosomes shrink greatly in size. When first they become visible, they are long and large (figure 8), but by the end of the period, they have become reduced to small, short, fat little bodies, all pretty much of a size, and not individually distinguishable (figures 14 and 15). There is bound to be a certain amount of unevenness in such a process of shrinkage. Some will contract a bit faster than others, perhaps one end of a chromosome somewhat faster than the other end. For a time it may look as though there is some recognizable difference in the size of the various chromosomes, but this difference soon disappears. The slight variations that can be detected during this period, therefore, seem to be neither constant nor significant.

The stage marks also the passing of the nucleolus. Plastered over against the nuclear membrane, and empty-looking except for a small black-staining endonucleolus, it begins to melt away, disappearing by insensible degrees.

The heterotypic metaphase and anaphase

When the chromosomes have ceased to shrink, and the nucleolus has disappeared, the nuclear membrane also dissolves away. Meanwhile, spindle fibers have made their appearance in the cytoplasm all around the nucleus, and these, as soon as the nuclear membrane has gone, penetrate to the interior, and surround and attach themselves to the chromosomes. At first, the spindle is multipolar, and the circles of chromosomes, still intact, lie without order or polarity (figures 14 and 15). Then the spindle becomes bipolar, and the circles begin to assume a definite position in the cell. I have already described this process in *Oe. franciscana sulfurea*. In the forms we are now considering, as in that one, the circles or chains do not as a rule break up at this time, but retain their identity throughout metaphase. The question at once arises as to whether it is possible for chromosomes, which come to the equatorial plate bound together into closed circles, to be subject to the laws of distribution which ordinarily prevail in organisms at this period. It is easy to see that chromosomes which are paired are almost certain to be separated to opposite poles in the heterotypic division, but when they are not paired, and all of them

Explanation of Plate 2

Figure 16.—Heterotypic metaphase, showing the regular zigzag arrangement of the chromosomes. Both circles are intact.

Figure 17.—The circle of 8 in heterotypic metaphase, regularly zigzag.

Figure 18.—The circle of 6 in heterotypic metaphase, regularly zigzag.

Figure 19.—An irregularity in the zigzag arrangement in a circle of 8.

Figure 20.—Heterotypic anaphase. Indications of the zigzag arrangement still persist. This is much more striking in the original than it can be made in a drawing, where all chromosomes have to be shown in one plane.

Figures 21, 22.—Chromosomes in anaphase, showing an early longitudinal split. Spindle fibers omitted.

Figure 23.—Telophase with early indications of the longitudinal split.

Figure 24.—The more usual appearance in telophase, in which the chromosomes have not yet revealed their split nature.

Figure 25.—Early interkinesis.

Figure 26.—Mid-interkinesis, the chromosomes in the form of Maltese crosses. Chromosome individuality is clearly maintained.

Figure 27.—Late interkinesis, just before the disappearance of the nuclear membranes. Both nuclei in the cell are shown.

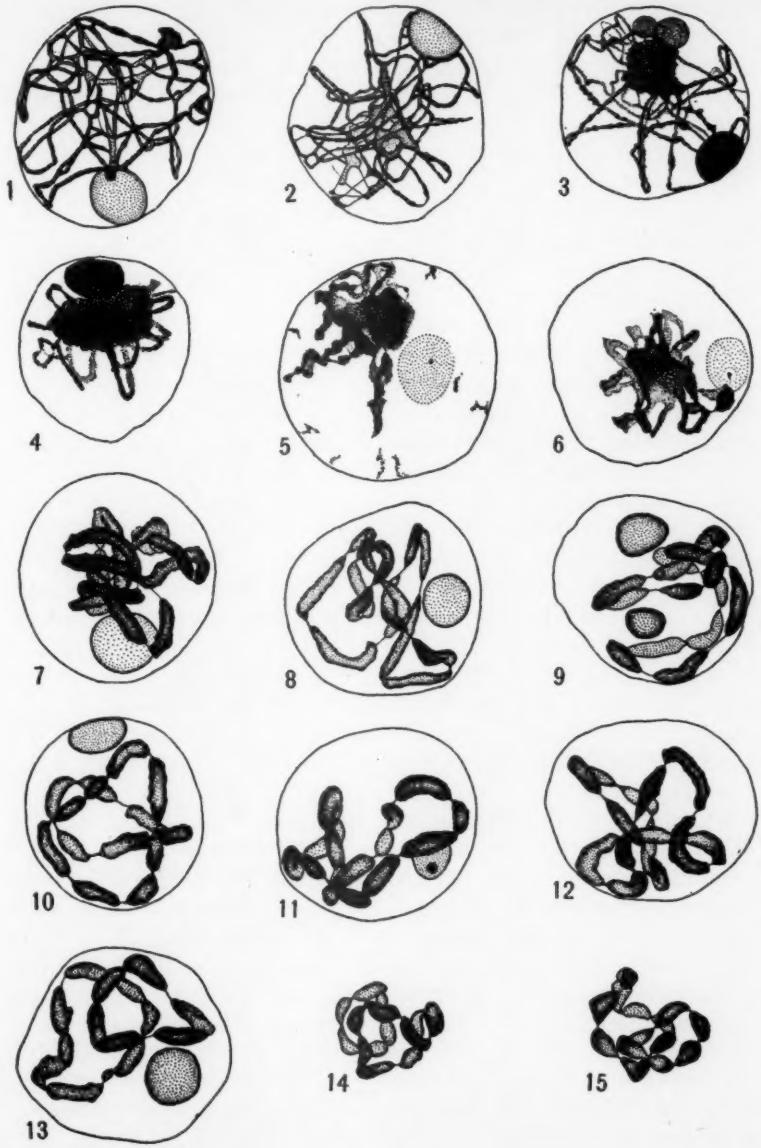
Figure 28.—The homoeotypic metaphase, showing both mitotic figures. The normal number of chromosomes present in each.

Figure 29.—The same. An unequal distribution of the chromosomes has occurred in the reduction division.

Figure 30.—The homoeotypic anaphase.

Figure 31.—Early telophase. Two of the four complexes shown.

Figures 32, 33, 34, 35.—The return of the granddaughter nucleus to the resting stage.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are more or less equally exposed to whatever influences may be at work between the equatorial region and the poles, it seems that we can hardly expect an entirely regular and uniform arrangement and separation. And yet, in *biennis* and *biennis sulfurea*, as in *Oe. franciscana sulfurea*, and other forms which I have studied, this seemingly improbable result is accomplished in a great majority of the cells, despite the entire absence of pairing of homologous chromosomes, and the fact that the univalents are still bound together. We find that in each circle, alternate chromosomes become attached by their middles to fibers leading to the same pole, and adjacent chromosomes, therefore, are in contact with fibers leading to opposite poles. As soon as these attachments are formed, the fibers appear to contract. The circles as a result take up a horizontal position across the equatorial region, and since adjacent chromosomes are being pulled toward opposite poles, we get the peculiar and very striking zigzag arrangement shown in figures 16 to 18. Even to the casual observer, the appearance in such a cell is one of regularity. Seven V-shaped chromosomes lie with their apices pointing to one pole, and seven of them point toward the other pole. Each set lies quite neatly in one plane, as seen from the side, and especially in late stages, when metaphase begins to give place to anaphase, a distinct space can be seen between the two sets, unoccupied by chromosomes. To the careful observer, this regularity becomes even more striking when the connections between the chromosomes are discovered, and the fact is noticed that those of one set tend to lie opposite the spaces between those of the other set. And so the chromosomes are for the most part regularly and equally distributed in these plants. As I have pointed out in discussing *Oe. franciscana sulfurea*, it is almost certain that they are so placed within the circles that the zigzag arrangement here described results in the actual separation of the members of every pair of homologous chromosomes.

There is a small percentage of cells in which irregularity in this chromosome arrangement occurs. Such irregularities may be described under three heads (see text-figures 1 to 3, also plate 2, figure 19): (1) In some cases, two adjacent chromosomes in one part of a circle are found to be drawn toward the same pole rather than toward opposite ones, and in another part of the circle, a corresponding pair are ready to pass to the other pole (text-figure 1). The number of chromosomes passing in either direction as a result of this irregularity will in all cases be normal. (2) Another type is where a pair of adjacent chromosomes, as before, are destined for the same pole, and at some other point in the circle, a chromosome is found uncertainly suspended across the equatorial region (text-

TEXT-FIGURES 1 to 3.—Diagrammatic views of the circle of 8 in heterotypic metaphase, with spindles omitted, to show observed types of irregularity in the zigzag arrangement of the chromosomes.

figure 2). This chromosome can apparently go to either pole. If it goes to the one to which the abnormally arranged pair goes, the number of chromosomes passing to that pole will be 8, and only 6 will go to the other. If it goes to the opposite pole, the number going to each pole will be normal, and the result will be the same as in the first type of irregularity. (3) A third sort of abnormal distribution is where two chromosomes in different parts of a circle are found to be suspended between the poles (text-figure 3). If both go to the same pole, 8 will pass to that one and 6 to the other. If they go to different poles, the number passing to each will be normal.

A careful study of some mid-interkinesis stages in *biennis* and *biennis sulfurea*, covering some 500 cells in the case of *biennis*, and over 600 cells in case of *biennis sulfurea*, showed that in about 2 to 5 percent of the cells, an unequal distribution had occurred, so that one nucleus received 8, and the other 6 chromosomes. No cases were observed in which the deviation from the normal number was greater than this, although if such irregularities as I have just mentioned occurred simultaneously in both circles, such might be possible. Since, however, irregularity in the zigzag arrangement of the chromosomes at metaphase does not necessarily result in unequal distribution, it follows that the amount of irregularity must be somewhat higher than the percentage here indicated. As I shall point out later, any irregularity in the zigzag arrangement will probably have an important genetical result, even though the number of chromosomes passing to the poles has not been changed from the normal.

In this connection, some data may be of interest, bearing upon the relative frequency with which the regular zigzag arrangement and deviations from it have been observed. Several slides of *Oe. biennis* were studied intensively with this end in view, every cell found in metaphase being examined carefully, except that the following classes of cells were eliminated from consideration: (1) Polar views, and other cells not affording a side view of the spindle. (2) Incomplete cells, and those in which the

chromosome arrangement was obviously disturbed by the knife. (3) Those in which the chromosomes were so badly clumped that nothing could be learned from them. All other cells were critically examined and the results tabulated under a number of headings, as seen in table 1.

TABLE 1

Analysis of a complete survey of the cells in heterotypic metaphase found on several slides of Oe. biennis.

CLASS	DESCRIPTION	NUMBER OF CELLS OBSERVED	PERCENTAGE
1	Both circles in the cell regularly zigzag	47	16.5
2	One circle clear and regularly zigzag, the other wholly or partially absent, or obscure	60	21.1
3	As much of both circles as could be seen, regularly zigzag	113	40.0
4	Mostly regular, with probably some irregularity	15	5.2
5	Obscure, with possible irregularities	20	7.0
6	Quite irregular, not clear	4	1.4
7	One circle regularly zigzag, the other not yet arranged (early metaphase)	5	1.76
8	Regularly zigzag in the main, with clearly observed irregularity of the types described in the text	16	5.6
9	Both circles clear, irregular	4	1.4

TABLE 2

Analysis of class 2 under table 1.

NUMBER OF CHROMOSOMES VISIBLE (ALL VISIBLE CHROMOSOMES REGULARLY ZIGZAG)	NUMBER OF CELLS
Circle of 8, and 5 of circle of 6	3
" " " " 4 " " " "	8
" " " " 3 " " " "	5
" " " " 2 " " " "	1
" " " " – – –	9
Circle of 6, and 7 of circle of 8	5
" " " " 6 " " " "	4
" " " " 5 " " " "	3
" " " " 4 " " " "	5
" " " " 3 " " " "	3
" " " " 2 " " " "	1
" " " " – – –	11
Chains of 6, and 2	1
" " " " 4	1
Total	60

TABLE 3

Analysis of class 3 under table 1.

NUMBER OF CHROMOSOMES IN EACH OF THE TWO CIRCLES CLEARLY OBSERVED TO BE ATTACHED END TO END, AND TO DISPLAY THE REGULAR ZIGZAG ARRANGEMENT	NUMBER OF CELLS
7 and 5	3
7 " 4	1
7 " 3	1
7 " –	6
6 " 5	3
6 " 4	8
6 " 3	5
6 " 2	1
6 " –	17
5 " 5	2
5 " 4	13
5 " 3	10
5 " –	10
4 " 4	10
4 " 3	14
4 " –	6

A further analysis of classes 2 and 3 may be desirable, and this I have presented in tables 2 and 3.

From the tables it will be seen that in a large number of the cells listed under classes 2 and 3, a great majority of the chromosomes were found to be regularly arranged. The proportion of these cells in which irregularities would have occurred is so slight as to be entirely negligible. The fact that classes 1 to 3 include 77.6 percent of the cells examined, and that 37.6 percent showed at least one regularly arranged circle in its entirety, proves rather convincingly that the zigzag arrangement in metaphase is a constant feature in these plants.

The circles do not break up until the chromosomes are pulled apart in anaphase. This phase lasts but a short time. The chromosomes are generally in the form of short V's as they pass to the poles (plate 2, figure 20), though in cases where there is an irregular arrangement in metaphase, some may pass to the poles as rods, the spindle fibers being attached near their ends. In most cases, no sign of a longitudinal split can be detected. In a few cells, however, its presence has been clearly indicated (figures 21 and 22), and it is probable that the chromosomes become split at this stage or earlier, but that in most cases the halves do not separate sufficiently at this time to make the fact evident.

Interkinesis and subsequent stages

The chromosomes, having arrived at the poles, are gathered together into a compact group and a nuclear membrane appears, enclosing them. When seven chromosomes are present, one tends to assume a central position, with the other six occupying the periphery (figure 24). The nucleus then grows rapidly in size until the maximum is reached. In the few cells in which the split nature of the chromosomes becomes evident in anaphase, they continue in early telophase to exhibit this characteristic (figure 23). In most cells, however, such a split has not as yet become visible. In these, the chromosomes lose at this time the V shape that characterized them during anaphase, and assume instead a more or less squarish appearance. They are very clearly defined, and at first have no connection with one another. About the time of the formation of the nuclear membrane, however, we notice that delicate little threads tend to pass out from the chromosomes, especially at the corners, and to fuse with one another, so that the chromosomes in the little cluster become slightly attached (figures 23 and 24). The formation of these threads is at once followed by an expansion of the chromosomes, which generally takes the form of an elongation and enlargement of the threads just formed (figures 25 and 26). Since these have developed at the corners as a rule, each chromosome soon begins to look like an irregular kind of cross. The chromosomes do not remain long attached, for the nucleus grows faster than they expand, and each chromosome tends to lie close to the nuclear membrane. Hence, the delicate threads by which they were joined soon break. If the expansion of the chromosomes were to keep pace with the growth of the nucleus, we might conceivably have developed a reticulum, but as it is, no reticulum is formed. At first the chromosomes are ragged, irregular structures, with the black-staining chromatin material unevenly scattered. When the nucleus has finally reached its maximum size, however, and the chromosomes their maximum degree of differentiation, they have a more definite shape. Each chromosome looks like a Maltese cross. The ends of the arms are swollen, and one or two additional swellings may be present in each arm. Usually the center of the chromosome looks solid. But in some, it can be clearly seen that there is a split running through the center. The chromosome is really a double affair, made up of two elongated parts. These have separated widely from each other at the ends, but they are still more or less intimately associated toward the center. Two of the arms, therefore, belong to one granddaughter chromosome, and two to the other.

There is some evidence at this stage to indicate that all of the chromosomes are not constructed in exactly the same manner. In some, the two halves are attached at the mid-region, and the two ends of each half are alike in size and shape. In others, the halves are attached at a region close to one end, so that one end of each half is about twice as long as the other. The difference between the two conditions is not very striking, so that unless one can study all seven chromosomes at once, it is not possible to say definitely whether it is at all significant. Unfortunately, on account of the fact that in every nucleus some of the crosses are seen edge on, it is not ordinarily possible to study a whole complex of chromosomes together, and so it cannot be stated definitely that visible structural differences in the chromosomes exist at this stage.

During early interkinesis, one or more nucleoli develop in each nucleus. These are small, round, and weakly staining, and invariably appear in contact with the chromosomes. They continue until the nucleus begins to shrink, then disappear. From their small size and empty appearance, it would seem that they are perhaps nearly if not quite functionless in these nuclei.

Judging from the frequency with which the stage is seen, mid-interkinesis lasts for a considerable period. In time, however, the chromosomes begin slowly to shrink, the X's becoming smaller and smaller; and finally the nucleus as a whole contracts rather quickly, crowding the chromosomes into a cluster at the center (figure 27). Then the nuclear membrane disappears, and the spindle fibers which have meanwhile formed in the cytoplasm penetrate and become attached to the chromosomes. At first the spindle is multipolar, but soon becomes bipolar, and the chromosomes are carried to the equatorial region and lined up in a regular fashion (figures 28 and 29). By this time, they have shrunken until they are very small indeed. Furthermore, the separation between the granddaughter chromosomes is often becoming very clearly marked. Each half resembles a small dumb-bell, since it is somewhat swollen at the ends, with a constriction in the middle. The two halves pair together perfectly during metaphase. The two homoeotypic spindles formed in the same cell may be in one plane or in different planes, often at right angles to each other.

The granddaughter chromosomes, as they pass to the poles, are regularly V-shaped, although sometimes they are in the form of rods (figure 30). At the poles, they are gathered into small clusters around which nuclear membranes are soon formed. As was the case in the heterotypic telophase, when seven chromosomes are present, one tends to occupy the center, with the other six surrounding it (figure 31).

As the four nuclei increase in size, the chromosomes undergo a process of elongation, until they reach a considerable length (figures 32 and 33). Then they begin to put forth delicate threads which anastomose, and form a loose reticulum. The chromatin material begins to move out into these threads, and little by little, a clear and prominent network is developed (figures 34 and 35). A large part of the chromatin material of each chromosome remains in one mass, but it becomes so irregular, and the material passing out into the threads forms other accumulations of such a nature that it soon becomes difficult, if not impossible, to distinguish the individual chromosomes or to tell what is the chromosome number. The return of the chromosomes to the reticulate condition in these cells does not conform to the processes as described in other plants by SHARP (1920), DIGBY (1910, 1919) and others. We have here a very clear formation of thread-like, anastomosing outgrowths from the chromosomes, which form the basis for the reticulum. This is quite different from the processes of internal expansion involving vacuolization, or splitting and attenuation of the chromosomes, such as described by these authors.

Nucleoli appear during this process, in contact with the chromosomes. At first they are small and weakly staining, but in time they attain to a fair size, and take the haematoxylin stain very strongly. They appear to arise *de novo*, from the material composing the chromosomes.

No cell walls are developed in the pollen mother cells of these plants until the granddaughter nuclei have been formed, and have passed more or less fully into the resting condition. The nuclei occupy positions near the periphery of the cell, and are about equidistant one from the other. The first sign of cell division is an invagination in the surface of the protoplast along lines midway between the nuclei. It is not until these invaginations have become quite large that cleavage planes appear. When they do appear, they apparently begin at the surface, and cut rapidly through to the center. Thus, the four pollen cells are formed simultaneously by a process of invagination followed by cleavage of the protoplast.

DISCUSSION

Cytological

Two facts of primary cytological interest have been emphasized in the cases of *Oe. franciscana* and *Oe. franciscana sulfurea* (CLELAND 1922, 1924). These are (a) the presence of telosynapsis, rather than parasynapsis, and (b) the regularity that characterizes the whole process of meiosis in the pollen mother cells.

(a) The cytologists who have worked on meiosis in the Oenotheras, with the exception of BOEDIJN (1924), have described the process as telosynaptic. The plants under discussion in this paper have proved no exception to the rule. Throughout all stages of the meiotic prophase in the anthers there is a lack of the sort of evidence that is necessary to prove the presence of parasynapsis. Neither pre-synizetic nor post-synizetic stages are characterized by parallelisms or splitting to any noticeable extent. This is particularly true of the open spireme stage. Added to these facts we have the evidence presented by second contraction and "diakinesis" stages. The radiating loops which appear in very early second contraction can be observed in every stage until the unfolding of the central mass, and the beginning of "diakinesis." No longitudinal splitting occurs in these loops as far as can be seen. They apparently become transformed directly into chromosomes which are strung end to end, and constitute parts of the spiremes which finally emerge. Thus, we can establish a clear relationship between the threads of the open spireme and those later, haploid spiremes which characterize the stage of "diakinesis." The very persistence of an end-to-end condition in "diakinesis" is in itself a strong piece of evidence in favor of telosynapsis. While it would be too much to say that the development of an arrangement such as is found here would not be possible under conditions of parasynapsis, it is extremely improbable that such a striking end-to-end arrangement of all 14 chromosomes would result, were the homologous chromosomes placed side by side in the open spireme. All of the evidence, therefore, seems to favor a telosynaptic interpretation in these plants.

(b) One of the most interesting things about meiosis in the Oenotheras is the regularity with which its processes are carried out, and this in spite of the fact that in many important respects they are quite different from the corresponding processes in most plants. The stages which are most at variance with what we find in other forms are diakinesis and the first maturation metaphase.

By diakinesis we usually mean a period during which the homologous chromosomes are closely paired, and the pairs widely separated from one another. This condition is altogether wanting in *biennis* and *biennis sulfurea*, there being in these forms an entire absence of pairing. In spite of this, however, we find no lack of uniformity. In all cells, with apparently few or no exceptions, the unpaired chromosomes are arranged end to end into closed circles, one of 6 and the other of 8 chromosomes, and the circles are as a rule linked together. The constant presence of such configurations as these is as striking as it is unusual. It probably means

that in earlier stages in prophase the network has a definite structure, such that when it condenses to form the spiremes, the individual chromosomes come out in the same way each time.

Nor is regularity of behavior confined, in all probability, to the arrangement of the chromosomes into two circles, each of a definite number. As I have pointed out in the case of *Oe. franciscana sulfurea*, there is little doubt that the chromosomes occupy definitely fixed positions within the circles also, for, with adjacent chromosomes going to opposite poles, the number of cells in which non-disjunction would occur if the chromosomes were placed in the circle by chance would be impossibly high.

In metaphase, the regularity of behavior is fully as striking as it is in the immediately preceding stage, perhaps more so, in view of its peculiarity. As in *Oe. franciscana sulfurea*, so here, we find that adjacent chromosomes are caught by fibers leading to opposite poles, and thus are separated in anaphase. I have already described the process. It remains only to emphasize the fact that the chromosomes are not brought to the equatorial region in a haphazard manner, and distributed apparently by chance, but the whole process from beginning to end is orderly and strictly according to rule, the occasional irregularities only serving to lay stress upon the conspicuous uniformity in behavior.

The orderly distribution of the chromosomes comprising the circles, to the poles, brings up the interesting question regarding the mechanism by which this is accomplished. We have a decidedly different problem here from that connected with the separation of half chromosomes in somatic mitoses, or of closely paired homologous chromosomes in meiosis. In neither of these cases does there appear to be any element of selection. In somatic mitoses, as far as we can see, either half of a split chromosome may pass to a given pole; and as for meiosis, aside from any cytological evidence supporting the theory that a given chromosome of a homologous pair may go to either pole, the laws of independent assortment of genes require that such be the case if the heterotypic division is to satisfy all requirements as the mechanism which is responsible for these laws. But in the case of the circles in Oenothera, the element of selection enters in. As a rule, every second chromosome is caught up on spindle fibers leading to a given pole, and carried to that pole. Chance apparently does not determine the direction in which a particular chromosome shall go, nor does proximity of the chromosomes to one pole or the other, as they lie in the newly formed bipolar spindle, have anything to do with it. There is some force of attraction between a given pole and certain chromosomes, or there is some other mechanism, as yet unexplained, which results

in the definite selection of a certain set to be taken to one pole, and another set to be carried to the other. Any theory, it seems to me, that attempts to explain the separation of chromosomes and their passage to the poles, must take into account this peculiar selection among the unpaired chromosomes of the Oenotheras.

Genetical

The probable reason for the failure of chromosomes to pair with their homologues in "diakinesis"

In *Oe. biennis* and *Oe. biennis sulfurea* not a single chromosome succeeds in pairing with its homologue. This is far from being a universal condition in the evening primroses, however; in fact, it is true in only three of the eleven species or varieties which I have thus far studied (CLELAND 1923, 1925). All conditions are found in the various species from complete absence of pairing, as here, to the presence of seven pairs, as in *Oe. blandina* and *Oe. deserens*. A given chromosome pair, then, does not necessarily behave in the same way in every species. In some, the individual chromosomes can pair, in others, they cannot. Why do chromosomes fail to pair in some species, and why do they often behave differently in the different species?

One might conceivably argue that the failure is due to the fact that the bonds of union between the telosynaptically arranged chromosomes are stronger than the tendency to pair, which tendency might even be considered as strong in the evening primroses as in other plants, but not strong enough to overcome the physical strength of the attaching threads. This seems to me to be untenable, however, for it cannot explain the regularity of chromosome arrangement in the various species which possess not only a circle, but a definite number of pairs as well; and it fails particularly in view of the fact that while in one species a certain set of homologues always break away from the spireme and pair up, in another species they are unable to do so.

At present I am holding to another theory, which seems to fit all the facts at my diposal. It is that the failure of the homologous chromosomes to pair is due to lack of affinity, and that this incompatibility between the homologues is the result of divergence in their constitutions. In other words, chromosomes which pair in diakinesis in Oenothera are relatively homozygous. Those which fail to pair are relatively heterozygous. Some of the reasons for adopting this theory are: (1) The only species that I have studied which have perfect pairing (*Oe. deserens* and *Oe. blandina*)

are entirely homozygous and have but one type of gamete. (2) *Oe. franciscana*, with its freedom from mutants, its uniform gametes, and its high percentage of seed and gamete fertility, has a large number of paired chromosomes, namely, 5 pairs, and a circle of 4 which in metaphase usually breaks into two pairs. (3) Some of the species that I have examined are known to be permanently heterozygous. All these have displayed circles, with partial to complete absence of paired chromosomes. (4) Using this theory as a basis, it is possible to explain the origin of certain mutants from parent forms. A discussion of this I shall reserve until a later paper. (5) The failure of homologous chromosomes to pair in diakinesis is characteristic of a good many hybrids, where there is every reason to believe that the corresponding chromosomes descended from the two parents are decidedly diverse in nature. The similarity between the cytological behavior in many Oenotheras and in such hybrids suggests that the lack of pairing in both is due to similar causes.

There is not room in this paper for a discussion of the possible origin of chromosome incompatibility in the Oenotheras. It may merely be stated at this time that it is perhaps not necessary to assume that it arose as the result of hybridization, but it may have come about in other ways, as for instance through the gradual accumulation of gene mutations within the chromosomes, a process aided probably by the appearance of balanced lethal factors. It may well be asked in this connection why a similar failure to pair has not commonly arisen in plants. We must assume either that gene mutations have for some reason taken place much more frequently in the evening primroses than in other organisms, or else that it requires fewer changes in an Oenothera chromosome to make it incompatible with its mate than is the case with the chromosomes in other plants. It may also be that it is more difficult for chromosomes to pair when they are arranged end to end, than it is when the homologues are already placed side by side in the spireme.

The genetic composition of *Oe. biennis* in relation to its chromosomes

Oe. biennis is one of the permanently heterozygous species of evening primrose. It appears to have two distinct types of gamete, but on account of the presence of lethal factors, the complexes carried by these cannot exist in the pure state and hence only the combination of the two survives. The result is a permanent heterozygote, which breeds true in the main. There seems to be a difference of opinion in regard to the nature of the lethal factors involved. RENNER (1917) found that both complexes can exist in the egg, but only one of them in the sperm. One of the complexes

would thus possess a sperm or pollen lethal, the other a zygote lethal. DE VRIES'S (1913) experiments, on the other hand, would indicate that there is but one kind of successful sperm, and one kind of egg, and that the kinds are different. One complex, then, would have a sperm or pollen lethal, the other an egg lethal. In either case, there is but one kind of successful pollen grain, or at any rate, the pollen gives rise to sperm of only one sort.

The diploid number of chromosomes in *Oe. biennis* is 14. Theoretically, species with this number ought to be able to produce as many as 2^7 different kinds of gametes, depending upon the number of pairs of homologues that happen to be heterozygous in a given individual. As few as two kinds of gamete could be produced under two conditions. First, a plant might have but one heterozygous pair, the other six pairs being homozygous. Second, all of the heterozygous chromosomes might be linked together and act as a unit in heredity. The difficulty with the first possibility is that the possession of only two kinds of gamete is characteristic, not of a small proportion of individuals, but, so far as we know, of all of them. It is unlikely that all plants of *Oe. biennis* are heterozygous in one and the same chromosome, and homozygous in the case of the other six. The other possibility seems *a priori* fully as unlikely. Nevertheless, the cytological evidence undoubtedly favors the second explanation. It is probably a fact that all of the chromosomes in *biennis* and *biennis sulfurea* are linked in such a way that they act as a unit in heredity.

I have already emphasized the probability that the chromosomes are arranged within the circles in a definite order, so that homologues are as a rule separated to opposite poles. This order probably applies not merely to the position of the pairs of homologues, but to that of individual chromosomes as well, so that each chromosome occupies a given position and does not ordinarily change position with any other chromosome, even with its homologue. Supposing this to be the situation, and recalling the fact that unpaired chromosomes are probably more or less heterozygous, let us call the four pairs which comprise the circle of eight AA′, BB′, CC′, and DD′; and let us assume that they are arranged within the circle in the following order—AA′BB′CC′DD′. In like manner, the circle of six can be lettered EE′FF′GG′. We know that, as a rule, adjacent chromosomes are separated and pass to opposite poles. In the circle of eight, therefore, ABCD will pass to one pole, and A′B′C′D′ to the other. Since the arrangement of chromosomes into circles seems to be constant, not only in all pollen mother cells of a given individual, but apparently in the various individuals of the species, we may conclude that

if in one cell chromosomes ABCD pass to one pole, and A′B′C′D′ to the other, they will be distributed after the same fashion in all normally behaving pollen mother cells throughout the species. This being the case, ABCD are as much linked in all pollen mother cells as though they were parts of a single chromosome, and the same applies to A′B′C′D′, EFG and E′F′G′. It is as though we had but two sets of homologues, then, instead of seven.

But if these two sets act independently, as do the various sets of homologous chromosomes in other plants, four kinds of gamete will be possible, ABCDEFG, ABCDE′F′G′, A′B′C′D′EFG and A′B′C′D′E′F′G′. This does not satisfy the genetical situation, for it has been shown that there are apparently but two types of microspore formed. In all of the other species that I have examined, in which there are unpaired chromosomes, these are grouped into but one circle, and the formation of only two kinds of gamete is easily explained. But *biennis* and *biennis sulfurea*, in having two circles, have proved exceptions to this rule, and an exact cytological explanation of the presence of but two types of gamete is therefore not so obvious. In order to bring chromosome behavior into line with genetical discoveries it is necessary to assume in the case of these species that a given chromosome complex resulting from one circle is under the necessity, ordinarily, of going to a given pole, along with a certain complex from the other circle. In other words, it is not a mere matter of chance whether EFG go to the same pole as ABCD or to the opposite one. When we stop to consider, this is what we would naturally expect in the circumstances. Chromosomes ABCD are apparently selected from the circle of eight and carried to one pole because there is some sort of attraction between them and that pole, and the other chromosomes are as much attracted by the other pole. The same applies to the circle of six. Each circle, then, has one set of chromosomes with affinities for each pole. Thus, we may say, for instance, that the complexes ABCD from one circle and EFG from the other have like affinities, and consequently pass to the same pole. But if this is true in one cell, it is no doubt true in all, for the complexes will surely possess the same characteristics in all normal pollen mother cells. The complexes from the two circles do not act independently, therefore, but the same chromosomes go to the same pole in all pollen mother cells, even though they be separated morphologically into two groups. Two complexes are formed as a result, which we shall letter ABCDEFG and A′B′C′D′E′F′G′. Only one of these complexes is able to survive in the sperm, however, owing to the presence of a lethal factor, so that but one kind of functional male

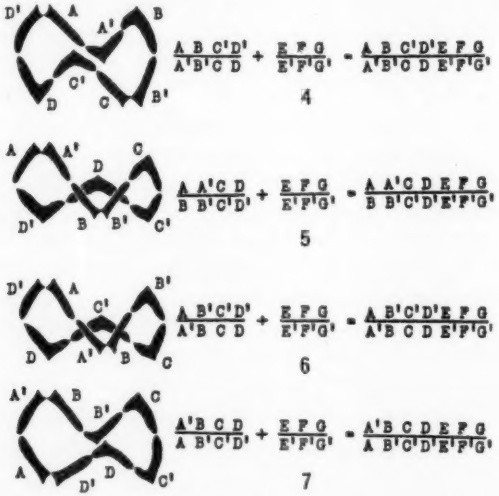
gamete is formed,—and that not because there is present in all cells but one heterozygous chromosome pair, but because all of the chromosomes are in effect grouped into one great linkage system, such that the same chromosomes go, as a rule, to the same pole in all cells, and form complexes corresponding to the two complexes which RENNER has called *rubens* and *albicans*. It can be said, then, that the genetical composition of *Oe. biennis* may apparently be explained on the basis of the behavior of the chromosomes during meiosis in the pollen mother cells, since the peculiar type of linkage which goes along with the presence of but two sets of gametes seems to be due to the way in which the chromosomes are linked, and then separated, in the heterotypic mitosis.

The genetical significance of irregularities in the zigzag arrangement of chromosomes

As I have already suggested, in a certain percentage of cases there is a departure from the regular zigzag arrangement of chromosomes in the heterotypic metaphase. I have already described three types of irregularity (text-figures 1 to 3) and brought out the fact that, as far as numerical distribution to the poles is concerned, these may in some cases result in abnormalities, although this is not necessarily the outcome. But even when irregularities of the various sorts do not cause a deviation from the number of chromosomes normally going to the poles, they nevertheless result in all cases in a distribution that is in other respects abnormal, as we shall see.

Wherever the number carried to the poles is 8 and 6, instead of the normal 7 and 7, the grains containing six chromosomes will probably prove functionless, or at least will give rise to functionless embryos. No 13-chromosome Oenotheras have ever been found, so far as I am aware. The 8-chromosome pollen grains, however, if they happen to possess the successful *rubens* complex, rather than the unsuccessful *albicans* one, may conceivably succeed in giving rise to individuals bearing 15 chromosomes, providing only that the extra chromosome which it has received is not the one possessing a lethal. If a successful 15-chromosome individual should arise, one of its sets of homologues would possess three members instead of two, and of these, two would belong to the *albicans* complex. Any recessive factors contained in the duplicated chromosome would probably express themselves under such circumstances, and a taxonomic character somewhat different from the normal might be displayed by the plant.

We have now to discuss the result of irregularities in *Oe. biennis* in cases where the number of chromosomes distributed to the poles is normal. I shall first suggest something of the variety of types of chromosome complex that can arise, and then indicate the probable genetical result of the formation of such complexes. I have taken for illustration the first of the three types of irregularity described in this paper. Several figures are presented herewith for purposes of illustration (text-figures 4 to 7). In all of the figures, the order in which the chromosomes are arranged within the circles has been kept the same, since, as I have stated before, I conceive this order to be constant.


D' A A' B D C' C B'
A B C'D' / A'B'C D + E F G / E'F'G' - A B C'D'E F G / A'B'C D E'F'G'
4
A A' D C D' B B' C'
A A'C D / B B'C'D' + E F G / E'F'G' - A A'C D E F G / B B'C'D'E'F'G'
5
D' A B' C' D A' B C
A B'C'D' / A'B C D + E F G / E'F'G' - A B'C'D'E F G / A'B C D E'F'G'
6
A' B C B' A D' D C'
A'B C D / A B'C'D' + E F G / E'F'G' - A'B C D E F G / A B'C'D'E'F'G'
7

TEXT-FIGURES 4 to 7.—Diagrams showing 4 of the 20 possible pairs of complexes that may arise when an irregularity of the type seen in text-figure 1 occurs in a circle of 8 chromosomes, while the circle of 6 is regularly distributed. Complexes resulting from the circle of 8 are shown immediately to the right of the diagrams, and the total complexes derived from both circles are given at the extreme right. Chromosomes above the horizontal lines go to the upper pole, those below the lines to the lower one.

In determining the total number of sets of complexes that can be formed as the result of the type of irregularity chosen, four factors which tend to increase the number must be taken into consideration. First, from the standpoint of their position in relation to the individual chromosomes, it is theoretically possible for the irregularities to occur at any point in the circle. Thus, in text-figures 5 and 6, the irregularities are identical in kind, but they have occurred at different points, and the

resulting complexes are therefore different. Second, the two abnormally arranged pairs may occupy different positions with reference to each other in the circles (compare text-figures 4 and 5). Third, since there are two circles in each cell, the position that the irregular complexes resulting from a circle occupy with reference to the poles must be taken into account. Thus, it will be noted that the complexes resulting from the circle of eight are alike in text-figures 6 and 7, but since they are carried to different poles in the two cases, they become associated with different chromosomes from the circle of six, and the total complexes are therefore different. Fourth, irregularities may occur in both circles in a cell, which greatly increases the number of complexes that are possible.

If one, taking these factors into consideration, will work out all of the possibilities, he will find that in the case of a circle of eight chromosomes the type of abnormality which we are discussing may result in as many as 20 different sets of complexes; and that in a circle of six, such an irregularity may give rise to any one of 12 different sets. In half of these sets (see text-figure 5), it will be found that one chromosome is entirely missing in each complex, whereas both members of another pair are present. Since none of these chromosome sets is complete, the probability is strong that many, if not all, will render the gametes bearing them incapable of functioning. For this reason I shall eliminate these cases from the discussion and deal only with those in which full complements are formed.

Cells in which the circle of 8 alone displays irregularity will give rise to any one of ten different sets of chromosome complexes in which both complexes will receive one member of every chromosome pair. Those in which such irregularity is confined to the circle of 6 can result similarly in any one of six different ways. Altogether, then, cells with irregularity in only one circle may give rise to pollen grains possessing any one of 16 different sets of complete chromosome complexes. But irregularities may occur in both circles at the same time. The total number of sets that are possible in such an event is the product of the numbers possible when the irregularities are confined to one circle, or $10 \times 6 = 60$ sets. The total number of sets of complexes possessing a full complement of chromosomes, and resulting from the type of irregularity that I have chosen, is therefore 76. One complex out of each set of two will probably fail to survive, since it will include the chromosome containing the sperm lethal. 76 types of sperm are therefore theoretically possible as the result of irregularities in one or both circles. These are all abnormal types of gamete, deviating in some way from the normal ABCDEFG type. Some of the complexes

resulting from such irregularities will differ from the normal in respect to only one chromosome (text-figure 7). Others will differ in respect to two or three chromosomes (text-figures 4 and 6). Greater deviation is not possible where only seven pairs are involved, and where each complex has a full complement of chromosomes.

We may now discuss the possible genetical significance of such irregularities in chromosome behavior. Suppose that we let the chromosome complex ABCDEFG represent the *rubens* gamete, and A′B′C′D′E′F′G′ the *albicans*. Suppose further that an irregularity such as is figured in text-figure 7 has occurred in a certain pollen mother cell, and that the pollen grains which result are among those used in a cross with another species. If A′ does not carry the *albicans* sperm lethal, and the sperm from these grains succeed in fertilizing eggs, the resulting plants will probably exhibit crossing over with respect to certain characters, for the factors contained in A′ will show up in these individuals instead of those contained in the normally present A. As we have seen, ABCDEFG are normally carried to the same pole in the heterotypic anaphase. Genes contained in these chromosomes are therefore linked in inheritance. Each chromosome is to the whole linkage system what a chromomere is to a single chromosome. In the case shown in text-figure 7 there has been an exchange of the members of one chromosome pair, comparable to the transfer of chromomeres or sections of chromomeres to corresponding positions in opposing chromosomes. The latter phenomenon is called crossing over. The former we will term "chromosome exchange." The result, in crosses, of crossing over and of chromosome exchange will be much alike, except that in the case of the interchromosomal type, more characters may be involved than would be the case if only a section of a certain chromosome pair were affected.

The result of interchromosomal crossing over in cases where the resulting pollen grains are used for selfing purposes, or for crossing between two plants of the same species, is worth noting. Using text-figure 7 again for illustration, when complex A′BCDEFG is used for purposes of self-pollination, an individual resulting from its union with the normal A′B′C′D′E′F′G′ complex will have two A′ chromosomes, but no A chromosome. Dominant characters, dependent upon genes in A will be lacking in this individual. Furthermore, the A′ chromosomes will undoubtedly be identical, both having been derived from the same plant. Any recessive characters that have lain hidden in A′ because of the fact that, in common with the rest of the complex, it normally cannot exist in the homozygous state, may now show out, and the probability is that in

one or more characters this individual may deviate somewhat from the normal run of *biennis* plants. All of this will be true, of course, only provided A′ does not possess a lethal factor. If a pollen grain containing this complex, instead of being used in selfing, is transferred to another plant, which, however, has been derived fairly recently from the same source, the two A′ chromosomes in the zygote will still undoubtedly be almost, if not entirely, identical and the same result may be expected. On the other hand, if the *biennis* plants that are used as parents are of widely different origins, the probability is that the two A′ chromosomes will be decidedly unlike, owing to the fact that gene mutations have been occurring in them, and have been accumulating, thanks to the enforced condition of heterozygosity; and these mutations will almost certainly have been different in the two cases. The likelihood of recessive characters being found in double dose, and showing out, will therefore be reduced.

From the cytological standpoint, we may expect to find that in any individuals where the two A′ chromosomes are almost or quite identical, these will leave the circle of 8, and pair up; but in individuals in which they are decidedly different they may fail to pair, and the circle of 8 will be formed as in normal individuals.

In cases where the number of chromosomes exchanged between the complexes is greater than one, the effect will be greater. A larger block of characters will show the effect of crossing over in crosses with other species, and the number of recessive characters that may show out as the result of self-pollination will be increased. In fact, it is conceivable that the number might be so large as to greatly modify the appearance of the resulting individual, and cause it to be regarded as a mutant. I believe that certain mutants in some species of Oenothera have probably arisen in just this way. Here, again, we should expect pairing of all homologous chromosomes which are identical in composition, so that an individual arising through self-pollination from the union of the complex AB′C′D′ EFG (text-figure 6) with the normal A′B′C′D′E′F′G′ complex would be expected to have three paired sets of homologues, B′B′, C′C′ and D′D′.

The chance that a given irregularity will occur in *biennis* is very small indeed. One of the three types of irregular arrangement in metaphase that I have mentioned can result only in the normal number of chromosomes going to the poles (text-figure 1). Two of the types, however, will probably result, in about 50 percent of the cases, in an unequal distribution. An examination of interkinesis stages shows that in about 2 to 5 percent of all the cells an unequal distribution has occurred. If the three types of irregularity that I have described (text-figures 1 to 3) are the

only three types, and if they occur with about the same frequency, those resulting in unequal distribution will be found in approximately one-third of the total number of cells in which irregularities have taken place. We may then estimate the proportion of cells in which the irregularities have failed to alter the number of chromosomes distributed as about 4 to 10 percent of the total number of pollen mother cells. In order to render the figuring simple, let us choose 8 percent as an approximate average of these. We have seen that there are 20 such irregularities possible in the circle of 8, and 12 in the circle of 6. The ratio of the possible irregularities in the two circles is 5 : 3. If the number of cells in which they occur totals about 8 percent of the whole, there will be about 5 chances in 100 that the circle of 8 will be irregularly arranged without altering the chromosome number in resultant cells, and about 3 chances in 100 that such irregularity will occur in the circle of 6. Each of the 20 possible irregularities in the circle of 8 will have one chance in 400 of appearing, and each of the 12 possibilities in the circle of 6 will also occur on the average in one cell in 400. The chance that irregularities will occur in both circles in the same cell is much smaller. There is only one chance in 660 of such an occurrence, and the probability of any particular combination of the possible irregularities in the two circles taking place is about one in 160,000; or in other words, it will appear on the average once in about 160,000 cells.

I do not claim, of course, that these figures are even approximately accurate, for I do not know whether there may not be more kinds of irregularity than the three that I have described, and I do not know the relative frequency with which these types occur. Certain conditions may favor the appearance of certain irregular chromosome complexes more often than others. Nevertheless it can be readily seen that the occurrence of any given irregularity is likely to be very rare indeed. While this is true of each individual irregularity, however, there will no doubt be a considerable proportion of cells that will experience some one of the numerous sorts of deviation capable of producing functional gametes. Approximately half of the sperm of normal chromosome number resulting from irregularities will have full complements of chromosomes and should prove functional. These will probably amount, therefore, to 3 or 4 percent of the total number of sperm, and consequently a number of plants in any given strain should show in some degree variations from the normal and uniform appearance.

Summing up the effect of irregular arrangements in metaphase, therefore, we may say that they cause the exchange of one or more chromosomes from one complex to the other, a phenomenon that I have called "inter-

chromosomal crossing over." Resultant reproductive cells which lack sperum lethals and have a complete set of chromosomes will probably prove functional, while all others will be incapable of functioning. In crosses with other species, the functioning of such gametes will result in the appearance of crossovers. In the case of self-pollination, the individuals resulting from the union of the modified complexes with normal gametes will vary from the normal in a degree depending upon the number of chromosomes which have crossed from one complex to the other. In the case of maximum transference, the resulting individual may perhaps be so different that it will be regarded as a mutant. The chances of such mutants appearing, however, are very slim. I have made no effort in this paper to calculate what might happen if a modified male gamete were to meet an egg likewise altered through the irregular distribution of the chromosomes. Not having studied meiosis in the embryo sac, I do not know, of course, that the same mechanism is found there.

The presence of chromosome exchange does not exclude in any way the possibility of the presence of crossing over of the usual type. In some cells in late second contraction, I have noticed that the appressed sides in the case of one or more of the peripheral loops have apparently become twisted about each other in a way that might conceivably result in the exchange of chromatin particles. With both types of crossing over cytologically possible, it would not be surprising to find cases of crossing over in *Oe. biennis* and *Oe. biennis sulfurea*.

The relation of *Oe. biennis sulfurea* to *Oe. biennis*

The arrangement of chromosomes in "diakinesis," and their behavior in metaphase are identical in the two forms. It can be safely assumed, therefore, that the *sulfurea* factor has arisen as the result of a gene mutation, rather than through chromosomal aberration. The foregoing discussion of *biennis* will therefore hold in all points for *biennis sulfurea*.

SUMMARY

Descriptive

1. In early prophase stages of both *Oe. biennis* and *Oe. biennis sulfurea*, the delicate network of the resting nucleus is transformed into a relatively thick-threaded, large-meshed reticulum, the so-called "open spireme." The process apparently involves the contraction of some threads, and their absorption into others; and the stresses and strains thus set up may be the cause of the synizetic contraction; or perhaps they do not in themselves cause shrinkage, but rather make it easy for the fixing fluid to

do so, in which case the contraction is to be regarded in the nature of an artifact.

2. The nucleoli, of which one is usually much larger than the others, are in intimate contact with the thread system, and probably contribute largely to the chromatin content of the network during this process. An endonucleolus becomes visible in late synizesis, and is frequently seen to have an organic connection with the reticulum.

3. With the approach of second contraction, a condensation of chromatin material occurs near the center of the nucleus, and peripheral loops become evident. These loops may be traced throughout the contraction stage, and when the knot unfolds, they are found to be parts of the spiremes which emerge.

4. In none of these stages do we find evidence of extended approximation of threads, or of splitting. The spireme is undoubtedly univalent in character, rather than bivalent, and the situation calls for a telosynaptic interpretation.

5. In late prophase, after second contraction, we find two closed circles, one composed of eight, and the other of six univalent chromosomes attached end to end. The two circles are linked, but may separate later. This arrangement is constant, and apparently characteristic of both of the species in question. Occasionally the circles are found to have broken open, forming chains of six or eight, rather than closed circles. In no cases do homologous chromosomes pair at this stage, as they do in diakinesis in most plants.

6. The circles usually remain intact throughout the heterotypic metaphase. After the formation of the bipolar spindle, alternate chromosomes become attached to spindle fibers leading to the same pole, adjacent chromosomes to fibers leading to opposite poles. In mid-metaphase, the circles lie horizontally across the equatorial region, and present a regular zigzag appearance, due to the fact that adjacent chromosomes are being pulled to opposite poles.

7. Irregularities in the zigzag arrangement are occasionally found, and have been described in the text.

8. Anaphases present for the most part a regular appearance. Seven chromosomes pass to each pole, except that in about 2 to 5 percent of the cases, eight go to one and six to the other, due to irregularities in the zigzag arrangement.

9. During interkinesis, the daughter nuclei increase in bulk, and the chromosomes become transformed into structures resembling Maltese crosses. Two of the arms of each cross belong to one granddaughter

chromosome, and two to the other, and the split between the two halves can be clearly seen in many cases. Chromosome individuality is clearly preserved throughout interkinesis.

10. The homoeotypic divisions occur simultaneously, the spindles lying in the same or in different planes. It is not until after the granddaughter nuclei have attained to the resting condition, and most traces of chromosome individuality have been lost, that walls are formed. The return of the chromosomes to the reticulate condition of the resting stage involves neither a process of splitting nor of vacuolization, but threads are put forth from neighboring chromosomes which amalgamate, and in this way a network is formed.

Discussion

11. While the behavior of the chromosomes during "diakinesis" and the heterotypic metaphase is quite out of keeping with what is found in most plants, it is in the main very regular and there is nothing hit-or-miss about it.

12. The chromosomes are undoubtedly arranged in a definite sequence within the circles, otherwise the amount of non-disjunction that would occur with adjacent chromosomes going to opposite poles, would be impossibly high.

13. Every chromosome seems to have some sort of affinity for a particular pole, otherwise the picking out of alternate chromosomes to be sent to the same pole is inexplicable. Each chromosome must go to one pole rather than the other, and as this applies to every chromosome in the cell, it naturally follows that in every cell the same chromosomes will go together to the poles. The effect will be the same as though the chromosomes were actually bound together structurally in such a way as to form one pair of large compound chromosomes.

14. The probable reason why homologous chromosomes fail to pair in "diakinesis" in the Oenotheras is because they are incompatible, and this incompatibility is presumably due to divergence in their constitutions. Relatively heterozygous chromosomes fail to pair, relatively homozygous ones are capable of pairing. In *biennis* and *biennis sulfurea*, therefore, all the chromosomes are probably relatively heterozygous.

15. *Oe. biennis* is a permanently heterozygous species, possessing but two recognizable types of gamete, which RENNER has called *rubens* and *albicans*. *Albicans* fails to survive in the sperm condition, so that all functional male gametes possess the *rubens* complex. In order that only two types of gamete shall be formed in a plant, all heterozygous genes

must be linked in one chromosome; or if they are in more than one, the chromosomes possessing them must be linked. In *biennis* and *biennis sulfurea* the fact that none of the homologous chromosomes are capable of pairing makes it seem probable that the heterozygous genes are distributed throughout all of them.

16. All the chromosomes, despite their being in two circles, will act in heredity as though they were linked, in view of the fact that alternate chromosomes are as a rule sent to the same pole, provided that they are arranged in a definite order within the circles, and that the reason for their being distributed as they are is because of an affinity or attraction of some sort between each chromosome and a given pole. Both of these suppositions appear to be justified, so that the conclusion is admissable as a working hypothesis. According to this theory, if pairs of homologous chromosomes are represented by AA′, BB′, CC′, etc., and if they are arranged within the two circles in the following manner: AA′BB′CC′DD′ and EE′FF′GG′, then, if chromosomes ABCD are attracted to the same pole as EFG in one cell, they will be so attracted in all normal pollen mother cells, and A′B′C′D′E′F′G′ will pass to the other pole. Only two chromosome complexes will be formed, and these will be the same as the complexes which united in the first place to form the plant. Lethal factors will make it impossible for any but a combination of the two complexes to survive, and hence, a permanently heterozygous species will result.

17. Irregularities in the zigzag arrangement of chromosomes at metaphase, such as are described in the text, will result in abnormally built complexes. Some of these will have eight chromosomes, and others only six. Still others will receive the normal number of chromosomes, in spite of the irregularity, but will nevertheless be incomplete, in that while they carry both members of one chromosome pair, they will lack entirely a representative of one of the other pairs. Of all these sorts of complex, the only ones likely successfully to function in reproduction are those with eight chromosomes.

18. Many complexes which are formed as the result of irregularity, however, will have the normal number of chromosomes, and a complete set of them; but from one to three of these will not belong normally to the complexes in which they find themselves, but rather to the opposite ones. Thus, for example, an irregular arrangement of a certain sort will yield the following complexes: A′BCDEFG′ and AB′C′D′E′F′G. The members of the homologous pairs AA′ and GG′ have gotten into the wrong complex. This phenomenon I have called "chromosome exchange"

to distinguish it from the ordinary kind of crossing over, which may perhaps also occur in these plants.

19. (a) When pollen grains arising from the last-mentioned type of irregularity are included among those used in crosses with other species, and the sperm developed from them function, the plants derived from these particular zygotes will exhibit crossing over with reference to the characters dependent upon genes which lie in the chromosomes which have crossed over into the wrong complex.

(b) In cases of self-pollination, plants arising through the functioning of such sperm with normal eggs will possess one or more chromosomes in duplicate, and if these are not the ones which possess zygote lethals, the plants should survive, and display certain new characters dependent upon recessive genes in those chromosomes which are now in the homozygous condition, as well as the lack of certain characters that are due to dominant genes in the corresponding chromosomes which are absent. The extent to which such plants will vary from the normal will depend upon the number of chromosomes which have exchanged places, and the consequent number of recessive characters which have therefore become evident, as well as the number of dominant characters which are lost. In rare cases, the plants may vary to such an extent as to warrant calling them mutants.

(c) Where two plants of the same species, but not traceable to a common self-pollinated ancestor are crossed, the chances of variations occurring when such a sperm fertilizes an egg will be considerably reduced, inasmuch as the occurrence and accumulation of gene mutations in the corresponding chromosomes of the two strains will have rendered these chromosomes less identical than in cases where both parents have had a relatively recent common origin.

20. The chances of a given irregularity, and hence of a given morphological variation occurring are very small.

21. *Oe. biennis sulfurea* is identical with *Oe. biennis* in its cytological behavior, so that the *sulfurea* factor has undoubtedly arisen as the result of a gene mutation.

In conclusion, it is a pleasure to acknowledge the kindness of Professor B. M. DAVIS, in permitting me to collect material in his experimental garden. To members of the botanical staff of the MARINE BIOLOGICAL LABORATORY, Woods Hole, Massachusetts, I am also indebted for laboratory facilities placed at my disposal during the summers of 1923 and 1924.

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DYNAMICS OF THE WAXY GENE IN MAIZE. I. THE CARBOHYDRATE RESERVES IN ENDOSPERM AND POLLEN[1]

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INTRODUCTION

The fact that the waxy factor in maize manifests its action in the pollen, that is, immediately following the reduction divisions, has suggested to us that favorable material might be found here for a study of the gene as a dynamic unit.

Up to the present time there has been no marked tendency in genetical research to go behind the superficial effects of the hereditary unit. There appear to be two main reasons for this; the elucidation of many important problems has not required it, and, secondly, in complex organisms such studies are beset with very considerable experimental difficulties. But in proving the genes the material bases of heredity and the controlling agents in development some of the most fundamental questions in biology have been transferred to these entities. Studies relating to the arrangement of genes in the chromosomes and the mechanics of their distribution have been highly successful, but the methods employed have not served in any commensurate degree to reveal the intimate nature of the factors

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or the manner in which these units function in ontogeny. In contemporary genetics the genes are essentially static elements. The advancement of our knowledge of them from obscure germinal constituents recognizable only by some admittedly partial effect on a somatic feature to dynamic units of known physico-chemical properties is an objective whose possibilities of attainment we may well examine.

There seems to be little hope, at this stage, of applying the methods of the constitutional chemist to this problem, so far as the particulate hereditary material itself is concerned. The isolation in pure form of any gene would appear to present insuperable difficulties, especially in view of the probable instability of such substances and the manifest difficulty of establishing their identity *in vitro*. The possibilities which may be involved here cannot yet be given serious consideration. It does not appear probable, moreover, that a study of the whole nucleus by the chemical methods at present available can shed much light on the properties the geneticist has found this organ to possess. In such a problem as the one in hand the chemist can render unique service, but his efforts will be more fruitful, in our opinion, if directed upon gene action rather than upon the genes themselves.

Since direct methods of inquiry are precluded, we are forced to study the gene through the effects it produces during development. The distribution of the hereditary units has been followed by the external modifications which they occasion in the organism, but little knowledge of the nature of these elements has been gained thereby. We must go deeper and attempt to trace out the chain of physiological processes which links the superficial changes to the hereditary factor. The nature of these reactions should afford some clues to the dynamic properties of the gene itself.

It is a common observation that a single factor may cause a variety of changes and the criticism might be made at the outset that this method of attack can never lead to a satisfactory comprehension of gene action on account of the sheer multiplicity of these effects. Even though under diverse circumstances, internal and external, different results are found, these do not necessarily imply different kinds of activity on the part of the gene. It may be that the primary effects of a single hereditary unit are definitely restricted and the manifold changes that we see may ensue as these basic activities impinge upon various other processes. To use a crude analogy we may compare the possible action of a gene to that of a pathogenic organism which, through the liberation of a specific toxin, causes a variety of alterations in the highly differentiated host which it invades.

Assuming then that many of the effects occasioned by the presence of a gene are secondary, our problem resolves itself into recognizing the primary ones and gaining access to them. In this we encounter real difficulties. The external changes which have led to the recognition of the factor may give no clue to the nature of the underlying processes. For various reasons, moreover, these processes may not be amenable to investigation. It will be seen, however, when we review the progress that has already been made in some lines that promise of success in such efforts is not wholly lacking.

In approaching this subject it should be borne in mind, however, that, unless we accept BATESON'S presence-and-absence hypothesis, an allelomorphic difference cannot be taken as indicative of the total action of a gene. On the assumption that both the dominant and recessive conditions have germinal representatives at the locus in question, our results would not bear upon the nature of either of these genes as whole units but upon the difference between them. We would be measuring, not what is added to the residual inheritance by one gene, but the differential action of two. On this latter basis a knowledge of the complete action of a gene would be possible only in so far as mutations were available, revealing all the potentialities of the original factor. Investigations such as the present may at best, therefore, reveal only a partial action of the gene. Similar studies on the members of a multiple allelomorphic series might afford a more complete picture.

The waxy character in maize, with which the present investigation deals, was first described by COLLINS (1909), who noted that it involved a new type of endosperm. The physical properties of the non-floury portions of this tissue resembled those of a hard wax, hence the name. In inheritance waxy behaves as a simple recessive to the non-waxy or normal condition. It was discovered by WEATHERWAX (1922) that the endosperm reserves form a reddish-brown compound with iodine, a fact which serves to distinguish this race from all other known varieties of maize.

Recent investigations by DEMEREC (1924), BRINK and MACGILLIVRAY (1924) and LONGLEY (1924) have demonstrated the action of the waxy gene in the pollen also. Whereas starch grains giving the typical blue reaction with iodine are formed in the pollen of other races, the granule in the pollen of waxy plants, although not staining as quickly, assume a reddish color when similarly treated. The most significant fact in this connection is that segregating plants produce the two kinds of pollen in equal proportions. This proves the activity of the non-waxy-waxy allelomorphs in the male gametophyte. In the vegetative tissues of the

plant, granules staining red with iodine have not been found, but blue-staining starch may be plentiful.

From the developmental standpoint the gametophyte in flowering plants is vastly simpler than the highly differentiated sporophyte. We are probably safe in assuming, in view of the facts now at hand, that the potentialities of the waxy gene, so far as fundamental changes in carbohydrate metabolism are concerned, at least, are as fully revealed in the pollen as in the diploid plant. If this is true, we may consider the waxy gene as conditioning primarily certain fundamental chemical reactions which are not dependent upon a high degree of differentiation of the tissues in which they occur. That is, the waxy gene appears to be concerned with basic metabolic processes and we may treat it without reference to the complications which surround its action in the much specialized sporophyte. This circumstance is of first importance in a study of the dynamics of the factor. At the same time the parallel effect in the seed furnishes an abundance of material for experimental investigation.

Previous studies have shown that the waxy gene occasions a modification in the kind of carbohydrate reserves laid down in the endosperm and pollen. We have found striking differences in enzyme activity in non-waxy and waxy plants, also, and it is to these two phases of the subject that our current investigations mainly relate. In this paper it is proposed to set forth the evidence regarding the above-mentioned carbohydrate reserves as a basis for the fuller consideration that can be given when the facts regarding enzyme differences are also examined.

We wish to acknowledge the aid rendered in these studies by Miss KATHLEEN DIETRICH, who has prepared the accompanying drawings and coöperated in certain of the experiments. Our thanks are due Miss MARGARET COLE for the faithful reproduction in plate 1 of the colors given by the non-waxy and waxy starches when treated with iodine.

CHEMICAL COMPOSITION OF POLLEN AND SEED

It has been stated by WEATHERWAX (1922) that the reserve material in the endosperm of the waxy maize consists of a single carbohydrate, erythrodextrin. The fact that this tissue gives a red color with the iodine-potassium-iodide solution does, indeed, suggest that dextrins may be the preponderating constituents, but chemical analyses do not bear this out. As table 1 shows, the principal reserve carbohydrate in the endosperm of waxy maize is starch. There is less than 2 percent of dextrins. These facts are of considerable interest.

The non-waxy seed used in our analyses came from a commercial variety of white dent known as Silver King, while the waxy grain was taken from an unrelated stock being used in pedigree-culture work. This diversity in origin might be considered objectionable when our aim is to distinguish between the effects of the non-waxy and waxy allelomorphs only, but the results of the analyses are so clear-cut in regard to the relative proportions of the three main classes of carbohydrates under consideration that this criticism is not important.

Three lots of non-waxy and waxy maize were analyzed. The first pair of samples comprised the dry seed; the second, grain which had been soaked in water for 24 hours at room temperature. The third lot consisted of three-day-old seedlings in which the plumules were one-quarter to one-half inch long. Since hydrolysis of the polysaccharides present is one of the chief reactions attending germination, it was believed that comparative analyses of this series should reveal any important differences in amount of the more readily available dextrins, if such difference existed in non-waxy and waxy maize. Only sugars were determined on the dry seed, but in the other two samples the amounts of starch and dextrin were measured also.

Except for the sugar determinations, in which the improvements suggested by SHAFFER and HARTMANN (1921) were adopted, the standard analytical methods approved by the ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS were used. It may assist in the interpretation of the results if the more important steps involved in the separation of the three main groups of carbohydrates are briefly indicated. Weighed amounts of the finely ground oven-dried material are extracted for 24 hours with water-free, alcohol-free ether to remove the fatty substances. Extraction of the residue with 90 percent alcohol boiled under a reflux condenser for 75 minutes brings the sugars into solution. The dextrins are then removed by a further extraction for 3 hours with 10 percent alcohol at room temperature and determined as dextrose after hydrolysis with 1 percent HCl. The sugar-free dextrin-free residue is then boiled, cooled to 38°C and digested with saliva until the solution gives no color with iodine, fresh saliva being added if necessary to complete the hydrolysis. The starch is likewise measured by the amount of dextrose formed. Separation of the dextrins and starch in the sugar-free material is dependent, therefore, upon the difference in solubility of these substances in 10 percent alcohol at ordinary temperatures. Let us now consider the relative amounts of these carbohydrates in the two classes of seed. The results are shown in table 1.

TABLE 1

Content of starch, dextrin and sugars in waxy and non-waxy maize. Values for carbohydrates are percentages based on dry weight.

SAMPLE	MOISTURE DETERMINATION		STARCH		DEXTRIN		SUGARS		
	Dry matter	Percent moisture	After 10 percent alcohol extraction	After water extraction	Extracted with 10 percent alcohol	Extracted with water	Reducing	Sucrose	Total
1[1] Non-waxy	91.94	8.06	.....	.....	....	....	0.18	1.71	1.89
Waxy	92.69	7.31	.....	.....	....	....	0.18	1.81	1.99
2[2] Non-waxy	94.57	5.43	57.85	62.83	0.90	1.28	0.28	1.14	1.42
Waxy	.....	....	59.93	62.68	1.87	1.53	0.31	1.39	1.70
3[3] Non-waxy	.....	....	62.97	.....	0.87	....	0.79	0.94	1.73
Waxy	94.71	5.29	60.18	.....	1.61	....	0.80	1.78	2.72

1[1] = dry seed; 2[2] = soaked 24 hours; 3[3] = germinated (plumules $\frac{1}{4}$ inch to $\frac{1}{2}$ inch long)

It is quite evident that these results are not consistent with the hypothesis that the principal reserve materials in waxy endosperm are dextrins. In sample 2, soaked for 24 hours, the content of "starch" in the waxy type is as great as that in the common variety and amounts to nearly 60 percent of the total substance. Furthermore, the amount of material separating out in the starch fraction of the waxy sample is over thirty times that appearing as dextrin. According to these analyses the waxy seed before germination contains about twice as much dextrin as the starchy, but the absolute quantities are very small in both cases, amounting to less than two percent. It is a point worthy of note that the samples taken during early germination do not show, in either case, an increase in dextrin content. With the possible exception of the dry seed, the waxy sort appears to contain somewhat larger amounts of sucrose than the non-waxy type.

Thinking that the low yield of dextrin from the variety staining red with iodine might be due to incomplete extraction, the process was continued for an equivalent period of time. No further dextrin was recovered from the non-waxy sample and the amount yielded by the waxy residue was so very small as to be negligible. Prolonged extraction of the sample soaked 24 hours with water in place of 10 percent alcohol only slightly altered the proportions of starch and dextrin, as the table shows.

Pollen was collected for analysis from four sets of plants, two of which were sister progenies derived, respectively, from the non-waxy and waxy seeds borne on a single self-pollinated ear. These latter stocks were designated R 1a and R 1b. In progeny R 1a the homozygous non-waxy plants only were used as the source of this class of pollen, the heterozygotes being excluded in the field on the basis of the color reaction of their pollen with iodine. We have found this test entirely reliable and very convenient for making this separation. Pollen was also gathered from a group of Rice popcorn plants and an unrelated waxy progeny.

Immediately following collection the pollen was dried in a vacuum oven for 24 hours at 40°C and then placed in a desiccator over concentrated sulfuric acid.

The results of the analyses are given in table 2. Starch determinations were made on the last-mentioned lots of pollen only. While waxy pollen does not show a blue reaction with iodine, it does contain about the same amount of starch as the non-waxy sample. The dextrin content of the two sorts of pollen is very similar, namely, about 2.25 percent, or roughly one-sixth the amount of starch, About one-half the total carbohydrates in the pollen, exclusive of celluloses, exists in the form of starch in both these classes of maize.

TABLE 2

Percentage composition of non-waxy and waxy pollen with respect to sugars, dextrin and starch. Results are calculated to moisture-free basis.

DESIGNATION	STARCH	DEXTRIN	REDUCING SUGARS	SUCROSE	TOTAL SUGARS
Non-waxy (Rice pop)	16.28	2.29	2.55	7.43	9.98
Waxy (unrelated)	14.96	2.16	1.95	8.93	10.88
Non-waxy (R 1a)	.....	2.19	2.20	8.59	10.79
Waxy (R 1b)	.....	2.33	0.95	11.32	12.27
Average non-waxy	16.28	2.25	2.37	7.81	10.27
Waxy	14.96	2.24	1.05	10.52	11.57

So far as the present results go, there appears to be a constant difference in the two kinds of pollen in the proportions of reducing sugars and sucrose, the non-waxy type containing less of the latter substance and more of the former than the waxy pollen. This may be of significance in relation to invertase activity.

The significant thing in these proximate analyses of the carbohydrates is the close similarity in the composition of the non-waxy and waxy types of maize. Both varieties contain about 60 percent of starch in the seed and about 15 percent in the pollen. Nor is there a very considerable difference in the relatively small amounts of dextrin present. On the basis of these quantitative determinations the principal reserve material in the pollen and endosperm of the waxy race is to be considered a starch and not a dextrin. One must be cautious, however, in classifying these polysaccharides about which so little is known as chemical individuals. Starches and dextrins may possibly merge into each other by imperceptible degrees and the distinction between certain of them may be rather arbitrary. For purposes of quantitative estimation, however, solubility in 10 percent alcohol or in water at ordinary temperatures is considered the distinguishing feature and on this basis we have no other recourse but to place the principal reserve material of waxy endosperm and pollen among the starches.

On the other hand the waxy reserves differ from ordinary starches in regard to a property which has been considered one of the most distinctive characteristics of this group of carbohydrates, namely, the ability to form a blue compound with iodine. There can be no question that the waxy starch differs from the common type. With a view of studying the nature of this difference we have prepared the two starches in pure form.

PREPARATION OF STARCHES IN PURE FORM FROM ENDOSPERM AND POLLEN

In the preparation of starch from the endosperm, whole corn was soaked for 12 hours at 60°C in a solution of sulfurous acid of specific gravity 1.007. The liquid was then run off and the softened grain beaten to a pulp in a large mortar. The embryos, the bran and any other coarser particles were then removed by washing on a wire sieve. The starchy portion was again crushed and kneaded through muslin of coarse mesh and the starch allowed to settle. This crude starch was purified by thoroughly agitating it in a 0.40 percent solution of sodium hydrate until the milky liquid assumed a greenish-yellow color. This addition of alkali assists in the removal of proteins and the remaining oil. After sufficient treatment with sodium hydroxide the separated starch and proteins were allowed to deposit and the supernatant solution was drawn off. The impure starch was then agitated with water and allowed to stand 20 minutes to permit the coagulated nitrogenous compounds to settle. The starch in suspension above was siphoned off and allowed to settle. After rewashing several times it was thoroughly dried. This method yields a refined product in satisfactory amounts.

It is much more difficult to prepare a clean sample of starch from the pollen. The starch grains are much smaller and the relatively large amounts of fatty substances are difficult to remove. A fairly pure product was obtained, however, in the following way. Five grams of pollen were extracted for 5 days with ether at room temperatures followed by absolute alcohol for 7 days. The alcohol was brought to 35°C and held for several hours during this period. After the alcohol was filtered off the pollen was mixed with clean quartz sand and a little water and ground in a mortar for about 30 minutes. The resulting paste was poured on a fine muslin cloth and kneaded in water until little further material passed the cloth. The suspension was allowed to settle for an hour, the supernatant liquid containing most of the starch then decanted off, leaving considerable sand behind. The starch was then collected by centrifuging for 5 minutes at 1800 r.p.m., but much fine sand was also thrown down. This mixture was partially separated by repeated centrifugation, but very small quartz particles remained as the principal impurity after drying.

FORM OF THE STARCH GRAINS FROM ENDOSPERM AND POLLEN

In order to secure strictly comparable material for these studies the waxy and non-waxy grains on a series of backcrossed ears were separated and the starches prepared from them according to the method described

above. Figure 1 is a photomicrograph of the starch grains from the non-waxy seeds. The waxy starch is shown in figure 2. Both these preparations were lightly stained with iodine. It is a noteworthy fact that endosperm reserves of the waxy variety are organized in granular form, as is the case in common maize. There is about the same variation in size of starch grain in the two types, but there appear to be small differences in contour.

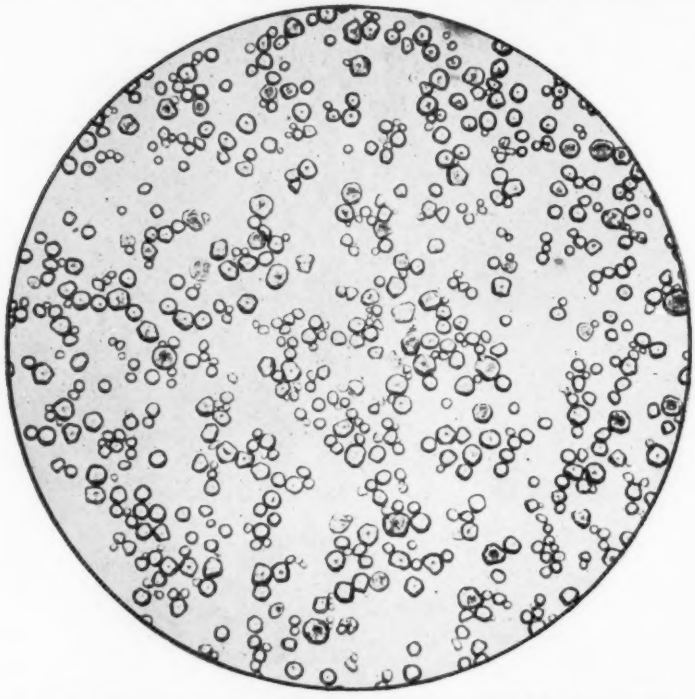

FIGURE 1.—Photomicrograph of the starch grains from non-waxy maize endosperm, lightly stained with iodine. Magnification ca. 250 diameters.

In general, the waxy grains are somewhat more angular than those of the other type. In both, the hilum is centric, but the non-waxy starch appears to be more finely fissured. It is possible that these relatively small morphological differences are due in part to the weak alkali used during preparation. There is a marked differential effect on the two classes of starch grains when treated with strong alkali or concentrated chloral hydrate, the shape of the waxy granules becoming quickly distorted. The swelling properties in water differ also.

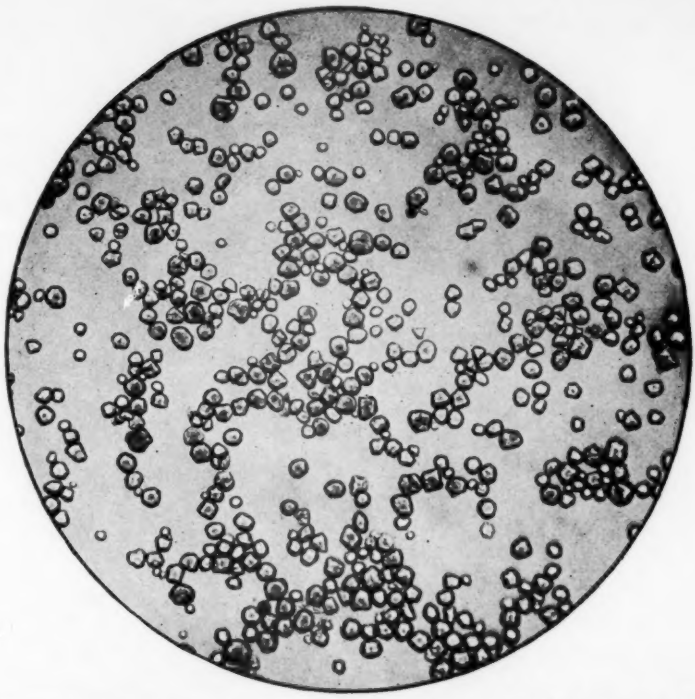

FIGURE 2.—Photomicrograph of the starch grains from waxy maize endosperm, lightly stained with iodine. Magnification ca. 250 diameters.

FIGURE 3.—Photomicrograph of starch grains from the pollen of non-waxy maize, heavily stained with iodine. Magnification ca. 900 diameters.

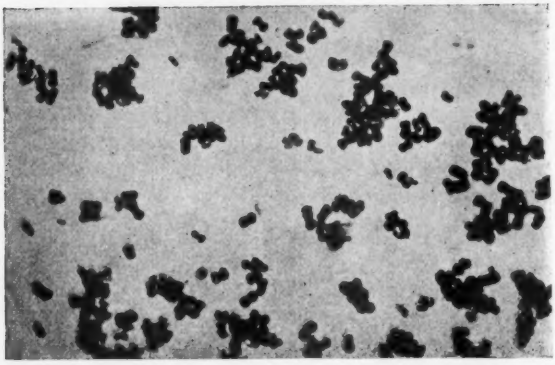

FIGURE 4.—Photomicrograph of starch grains from the pollen of waxy maize, heavily stained with iodine. Magnification ca. 900 diameters.

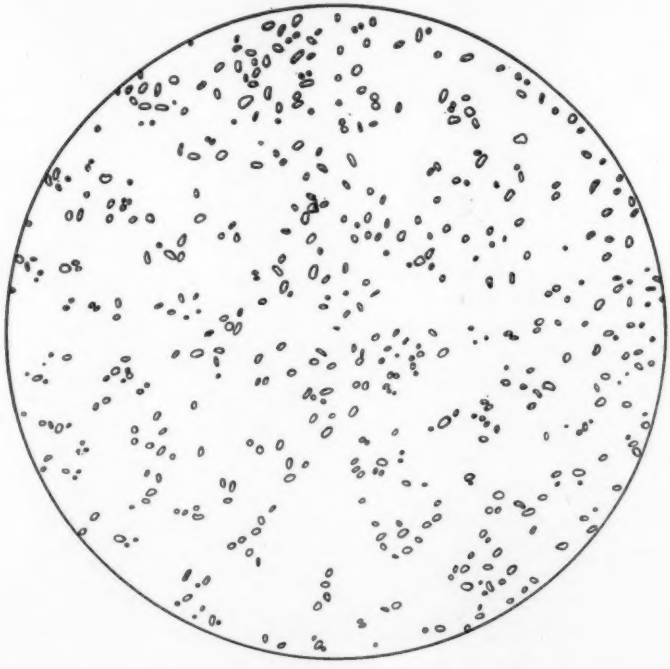

FIGURE 5.—Drawing of starch grains from the pollen of non-waxy maize. Magnification ca. 600 diameters.

In the pollen the starch grains are much smaller, seldom measuring more than $4\mu \times 1.5\mu$ through their main axes. As shown in figure 3 the granules from non-waxy pollen are quite regularly ovoid in shape. In waxy pollen, on the other hand, the grains are often dumb-bell-shaped, although, as may be seen from the photograph reproduced as figure 4, their form is less regular than in the former case. The differences between

FIGURE 6.—Drawing of starch grains from the pollen of waxy maize. Magnification ca. 600 diameters.

the starches in the two types of pollen are more clearly brought out in the drawings shown in figures 5 and 6. There is unquestionably a real morphological difference in the pollen starch grains of the waxy and non-waxy races of maize.

It is noteworthy that the waxy granules do not show the etching characteristic of the starch grains in the sugary race of maize and the wrinkled variety of the pea.

When examined under polarized light the starch grains from the endosperm of non-waxy and waxy maize show no marked differences. In

LEGEND FOR PLATE 1

FIGURE A.—Color reaction of aqueous suspensions of gelatinized pollen and endosperm starch from non-waxy maize with iodine-potassium-iodide.

FIGURE B.—Color reaction of aqueous suspensions of gelatinized pollen and endosperm starch from waxy maize with iodine-potassium-iodide.

FIGURE A

FIGURE B

both types the figure is usually centric and the lines are commonly at right angles to each other. The bands are possibly more clear-cut in the case of the waxy granules.

COLOR REACTIONS OF THE ENDOSPERM AND POLLEN STARCHES WITH IODINE

The most striking difference in the starches from waxy and non-waxy seeds is shown on treatment with the common iodine reagent (1.0 percent potassium iodide, 0.3 percent iodine in aqueous solution). Starch from the endosperm of ordinary maize, in common with that from nearly every other source, produces an intense blue color under these conditions. This result is so generally characteristic that it has come to be known as the "starch reaction." The starch grains in the endosperm of waxy maize, however, form a reddish-brown compound with iodine. The solutions prepared by boiling 0.1 percent of these two starches with water likewise react very differently to this test. If to 10 cc samples 5 drops of the above reagent be added, the ordinary starch gives a color described by RIDGWAY (1912) as Helvetia Blue, whereas that of the waxy starch is Rood's Violet. The concentration of starch and iodine may be widely varied without obliterating the difference.

The starch grains from the pollen of common maize also form a deep blue compound immediately on being treated with iodine. This reaction is not obtained with starch from waxy pollen. These latter do not stain at all when the reagent is first applied, but if the solution is concentrated by evaporation of the water and renewed from time to time, a reddish color is formed. The identification of non-waxy and waxy pollen (DEMEREC 1924; BRINK and MACGILLIVRAY 1924) rests upon this differential color reaction of the respective types of starch grains with iodine. If small amounts of the pollen starches are boiled and a few drops of iodine added to the suspensions after cooling, the same color reactions are obtained as with the corresponding endosperm reserves, namely, Helvetia Blue and Rood's Violet. In plate 1, figure A, is shown the color reaction with iodine of starch from the pollen and endosperm of non-waxy maize; figure B illustrates the color obtained on similarly treating he pollen and endosperm starches of waxy maize.

The identity of the color reactions with iodine and the similarity shown in rate of hydrolysis with amylase, to be discussed below, make it seem probable that there is a close correspondence in chemical composition between the pollen and endosperm starches in the two respective classes of maize. Their resemblance with respect to this property is good evidence

for the view that the same fundamental carbohydrate transformations occur in the pollen, which is an independent and relatively simple, binucleate structure, as in the endosperm, where the accessory conditions are more complex. As we have pointed out above, this circumstance should make the basic physiological action of the waxy gene more accessible to investigation.

COMPOSITION OF THE STARCH GRAIN

Agreement regarding the composition of the starch grain has by no means been reached. The literature pertaining to the subject is very large and the reader who desires an exhaustive treatment is referred to the summaries found in CZAPEK (1913) and especially ABDERHALDEN (1923). We shall limit ourselves here to a few of the more pertinent contributions.

The idea that the starch grain is not composed of a single homogeneous substance has received support from numerous investigations. NÄGELI (1858) distinguished two main constituents, granulose, the more soluble portion responsible for the blue color with iodine, and starch cellulose which, he considered, formed the skeleton of the grain. MAQUENNE and ROUX (1903, 1905) contend that the principal constituent of the starch grain is amylose, a substance which gives a blue reaction with iodine and exists in two polymeric forms, one of which is soluble in hot water and the other in superheated steam. A second substance, which they term amylopectin, is mainly responsible for the viscosity of starch pastes. It swells in hot water but does not dissolve, and is incompletely hydrolyzed to sugars with diastase. MEYER (1895) had earlier described two similar substances which he termed β-amylose and α-amylose, respectively. BIEDERMANN (1920) distinguishes a third substance called amylocellulose, which remains after treatment of amylopectin with salivary diastase. LING and NANJI (1923) have also found a substance in the starch of wheat, barley and rice, that behaves like a hemicellulose. In contrast with these views, BOURQUELOT (1887) assumes that the starch grain is composed of a large number of closely related carbohydrates.

SHERMAN and BAKER (1916) have shown that when 1 percent raw starch is gelatinized in water at 85°C and centrifuged at the rate of 2400 r.p.m. for 30 minutes it is separated into a lighter, clearer, more limpid layer containing the more soluble constituent (β-amylose of MEYER, amylose of MAQUENNE and ROUX) and a heavier more viscous layer containing the less soluble component (α-amylose of MEYER, amylopectin of MAQUENNE and ROUX). The presence of 0.001 M NaCl is necessary

in order to effect the partition. SHERMAN and BAKER found that potato starch treated in this way yielded approximately 20 percent of β-amylose, to adopt MEYER'S terminology. Some important differences in the properties of the two fractions were established. It required from 13 to 18 times as much of the twice-washed residual gel (crude α-amylose) to produce a given intensity of blue color with iodine as was required of the β-amylose. The β-amylose was found to be unstable, rapidly changing to the insoluble form even when stored in a refrigerator. With malt amylose the β-fraction is more readily hydrolyzed to compounds giving no reaction with iodine than is the α-fraction.

The starches prepared from waxy and non-waxy maize differ very greatly in the proportions of α- and β-amylose which they contain. After some preliminary attempts to separate these constituents, the following method, which differs from that of SHERMAN and BAKER only in that no effort was made to purify the products obtained from the first centrifugation, was adopted. Two-gram samples of each of the starches were heated in 100 cc of water for 10 minutes at 85°C. After adding enough tenth-normal NaCl to make the suspensions 0.002 M, they were centrifuged for 30 minutes at 2400 r.p.m. The non-waxy type of starch under this treatment separates into two distinct layers, as SHERMAN and BAKER found with potato-starch suspensions, the upper one being relatively clear and the lower very thick and slimy.

TABLE 3

Relative amounts of crude α- and β-amylose in the endosperm starches from non-waxy and waxy maize.

	TEST 1		TEST 2	
	Non-waxy	Waxy	Non-waxy	Waxy
Total yield in grams of crude β-amylose from 2 grams of starch	0.1587	1.2317	0.1587	1.1934
Percent of crude β-amylose	8.0	61.6	8.0	59.7
Percent of crude α-amylose by difference	92.0	38.4	92.0	40.3

The starch from the waxy type of grain as shown in figure 7 gives a quite different picture in the centrifuge tube. While the line of demarkation is nearly as well defined, the relative volumes occupied by the two layers and their appearance are very different. The upper layer in the

waxy tube is not as deep as that in the non-waxy one, but in the former the concentration of the more soluble β-amylose is so much greater that it gives this stratum a heavy opalescent appearance. Moreover, the lower layer in the waxy tube is less viscous than the α-amylose fraction from the non-waxy starch.

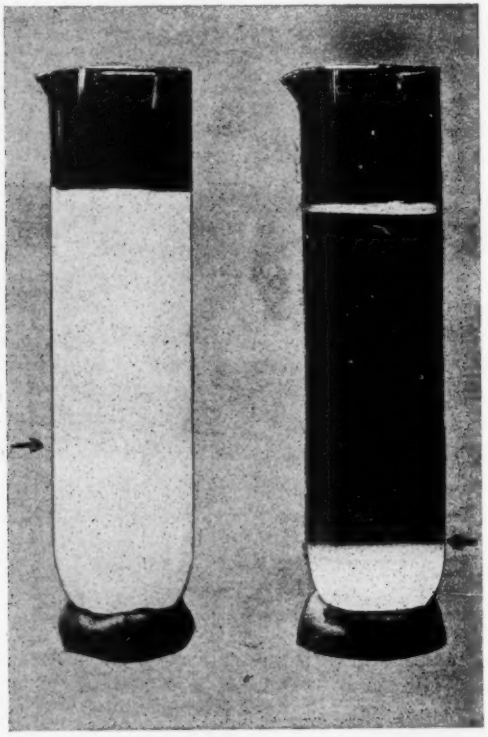

FIGURE 7.—Photographs of one percent suspensions of waxy starch (left) and non-waxy starch (right) after centrifugation for 30 minutes at 2400 r.p.m. The arrows point to the junctions of the layers of α- and β-amylose.

The supernatant layers containing the β-fractions were pipetted off and their volumes measured. To determine the amounts of starch present, 50 cc samples were concentrated on a steam bath and dried over night in an oven at 100°C and weighed. Table 3 shows the relative proportions of crude α- and β-amylose in the two kinds of starches.

The results of the two tests made are in close agreement.[2] The waxy type of starch contains about seven and one-half times as much of the more soluble constituent as the sample prepared from the non-waxy seeds borne on the same ears.

It is not to be inferred, however, that the α-fractions or the β-fractions are chemically identical in the two cases and that the differences in the two kinds of starches are explicable on the assumption that they are composed of the same two substances but in different proportions. When tested with iodine, both constituents of the waxy starch show the color described by RIDGWAY (1912) as Rood's Violet, whereas the preparations from the non-waxy starch stain the familiar blue. It is a significant fact in support of the view that the main reserve material of the waxy endosperm is a starch and not a dextrin, that the waxy-type granules comprise two substances which separate under the action of centrifugal force in the same way as the α- and β-amyloses of the common starches. But, as indicated by their iodine reactions and their behavior under the action of hydrolytic agents discussed below, there is some fundamental difference in these amyloses from non-waxy and waxy starches, on which the present experiment sheds no light.

RATE OF HYDROLYSIS OF NON-WAXY AND WAXY STARCH WITH HCl AND AMYLASE

Experiments were made to determine if the starches from non-waxy and waxy seeds were hydrolyzed at different rates under the action of dilute HCl and of salivary amylase. As a measure of the rate of digestion, the time required for the solution to reach the stage at which no color was given with iodine was taken.

The procedure with an acid hydrolysis was briefly as follows: 2-gram samples of each of the two sorts of starch were weighed out and boiled with 100 cc of water for 5 minutes. The volumes were brought to 96 cc and the solutions transferred to 500 cc Erlenmeyer flasks. These were fitted with reflux condensers and placed in a water bath. After bringing the liquid to boiling temperature, 4 cc of 25 percent HCl solution were added to each flask, giving a concentration of hydrochloric acid of 1 percent. A delivery tube was arranged so that samples could be forced

[2] The two determinations were not carried out at the same time, so the identical values for total yield of β-amylose from the non-waxy type in tests 1 and 2 are not to be explained by assuming that the same sample was weighed twice. There was either a mistake in our records or the case is an unusual coincidence. But even if the results of the one or the other test are thrown out, the main point is clear.

TABLE 4

Rate of hydrolysis of non-waxy and waxy starch paste with 1 percent HCl.

TEST 1			TEST 2		
Time in minutes required for hydrolysis		Ratio of times, waxy = 1	Time in minutes required for hydrolysis		Ratio of times, waxy = 1
Non-waxy	Waxy		Non-waxy	Waxy	
45	30	1.50 : 1	53	32	1.65 : 1

out periodically by means of air pressure without opening the flasks. This made it possible to maintain a constant temperature and prevented changes in concentration of the reagents. During the early stages of the digestion color tests against a background of white tile were made at 10-minute intervals and as the reaction approached completion, at 5-minute and 2-minute periods.

The results in table 4 show that the waxy starch is hydrolyzed to compounds giving no color with iodine, about one and one-half times as rapidly as the non-waxy starch. The differences in time in tests 1 and 2 are due in part to the difficulty of distinguishing the end point exactly, although the limits within which one can be sure this falls are relatively narrow. It is clear from the results obtained in these experiments that the waxy starch is less resistant to acid hydrolysis than the non-waxy starch.

The difference in rate of hydrolysis of the two kinds of starch with salivary amylase is very much greater than with acid. In fact, the disparity is so large that it is difficult to arrange a satisfactory comparative test. The amount of saliva necessary to carry the digestion of non-waxy starch to completion will hydrolyze the same quantity of waxy starch so rapidly that the rate cannot be measured. If the saliva is reduced sufficiently to enable the hydrolysis of the waxy sample to be accurately followed, digestion of the non-waxy sample is only partial, considerable starch remaining even after some hours.

After several fruitless attempts to measure the rates of these reactions by using different amounts of enzyme for the two kinds of starches, two experiments were made which were considered fairly satisfactory so far as opportunity to time the reaction and sharpness of end point were concerned. It was not found possible, however, to get as accurate results as in the tests on acid hydrolysis. The starch was boiled 5 minutes and diluted to 0.25 percent. The digestions were carried out on a water bath at 40°C. In the first experiment 5 cc of undiluted saliva were added to

50 cc of the solution of non-waxy starch. For the hydrolysis of the waxy starch the saliva was first diluted 1 to 4 and only 2.5 cc were used, added to the same volume of solution. The latter sample, therefore, contained one-eighth the amount of enzyme in the former. If we assume, as a first approximation, that the rate of hydrolysis is a linear function of concentration of enzyme, the time required for digestion of the non-waxy starch should be multiplied by 8 to make it comparable with that for the waxy sample. The result given in table 5 under test 1 indicates that the waxy starch is hydrolyzed over one hundred times as rapidly with salivary amylase as is the non-waxy starch.

TABLE 5

Rate of hydrolysis of non-waxy and waxy starch pastes with salivary amylase.

TEST 1			TEST 2		
Time in minutes required for hydrolysis		Ratio of times, waxy = 1	Time in minutes required for hydrolysis		Ratio of times, waxy = 1
Non-waxy	Waxy		Non-waxy	Waxy	
45×8*	3	120 : 1	16×16*	4	64 : 1

* Correction factors for differences in concentration of enzyme.

In test 2 the same amounts of starch were employed, but 16 times as much enzyme was used in the non-waxy sample as in the waxy. The end point with the solution of non-waxy starch was taken somewhat earlier than in the first test, namely, when the color with iodine was passing from olive to yellow. A little starch undoubtedly remains at this stage, but the rate of disappearance of the last traces is so low that a later reading must be rather arbitrary. The ratio of times in this test, when corrected for the difference in amount of enzyme, was found to be 64 : 1.

A remarkable difference in ease of hydrolysis of non-waxy and waxy starch with saliva is thus demonstrated. It seems probable that this difference is shown by both the α and β components. We have not tested these substrates with saliva, but some other experiments with amylase preparations from non-waxy and waxy seedlings on the two β-amyloses, respectively, although not made with this particular point in mind at the time, shed some light on the question. The concentrations of starch in the solutions tested were only approximately known, but it appeared that the waxy β-amylose was hydrolyzed about 3 times as rapidly as the corresponding substance from non-waxy starch. While the amount of the difference may be subject to revision it seems clear that the waxy β-

amylose is broken down more rapidly. A difference of this order, however, will not explain the great disparity in rates of hydrolysis of the whole starches. This is to be attributed mainly, we believe, to the dissimilarity in digestibility of the α-amyloses, this fraction from waxy starch being more readily hydrolyzed.

Experiments on the rate of hydrolysis of the α-amyloses themselves are unsatisfactory, since, with ordinary amounts of enzyme the reaction does not go to completion. The presence of an undigested residue obscures any earlier end point that might be taken.

Tests on rate of hydrolysis of the pollen starches with salivary amylase show that the waxy type is more readily digested than the non-waxy sort. Due to the impurities (principally fine quartz sand) in our preparations, it was not possible to put these experiments on a satisfactory quantitative basis. The difference in times required for disappearance of color with iodine in the two cases were so large, however, that there can be no doubt of the fact that the red-staining starch is broken down much more speedily than that from non-waxy pollen.

DIGESTION OF THE ENDOSPERM RESERVES DURING SEEDLING DEVELOPMENT AND RATE OF GROWTH IN THE SPROUT

In view of the relative ease with which waxy starch is hydrolyzed by amylase, it might be expected that during germination the endosperm reserves of this form as compared with those of the non-waxy type would be more quickly digested, and that the seedlings would make a more rapid growth. This does not prove to be the case, however. The endosperm of the non-waxy variety is utilized nearly as rapidly as that in waxy maize, and the two classes of sprouts make about equal gains in weight during the seedling stage.

In the experiments which led to these conclusions non-waxy and waxy seedlings were grown in the dark under conditions of controlled temperature and soil moisture and the changes in dry weight of the seed residue and the sprouts followed. In the first test it was found, on collecting the initial samples, that about 40 percent of the stored materials in the endosperm had already been removed. The experiment was therefore repeated, collections being made frequently enough to follow the whole course of seedling development. The seed used came from 4 carefully selected ears which had resulted from backcrossing heterozygous non-waxy plants with the waxy type. The grains from the butts and tips of the ears and any obviously defective individuals were discarded. Eight lots of 55 seeds each of the non-waxy and waxy types, respectively, were counted out

TABLE 6

Decrease in weight of endosperm of non-waxy and waxy grains during seedling growth. The values given are relative weights of the respective residues, the check samples being taken as 100.

AGE OF SEEDLINGS IN DAYS	WEIGHT OF SEED RESIDUES	
	Non-waxy	Waxy
0	100.0	100.0
1	100.4	98.0
2	93.7	93.0
3	78.2	81.3
4	68.0	70.1
6	39.5	34.4
8	23.4	21.4
9	21.5	18.0

and by substitutions of smaller grains for larger or *vice versa*, each sample was adjusted to weigh 12.0 grams. After soaking in water for 24 hours the seeds were planted in clean quartz sand approximately 50 percent saturated with moisture in galvanized iron boxes 13 inches by 4 inches by 4 inches. In each of these containers two 55-seed samples, a non-waxy and a waxy, were planted, the groups being separated by a movable center strip running from end to end. The boxes were weighed and placed in a darkened incubator set at 30°C. Throughout the course of the experiment the water lost by evaporation was replaced daily. Arrangement of the test in this fashion made the conditions under which the two classes of seed were grown closely comparable.

Samples consisting of the two lots of seedlings in a box were collected at the intervals given in table 6. After the sand was washed out of the roots with as little loss of tissue as possible, the sprouts and the residues of the seeds were separated at once. While the plants are fresh this operation can be performed cleanly, the adhesion of the embryo to the seed residue being very slight. The separate tissues were then dried at 100°C for at least 15 hours and weighed.

Up to the third day all the seeds or seedlings were included in the weighed samples, but thereafter the ungerminated grains were discarded. Five lots comprised fewer than 50 individuals, the lowest number being 41. In computing the values given in tables 6 and 7, corrections for variations in number were made by adjusting the weights to a basis of 50 seedlings.

The data given in table 6 are plotted in figure 8. It is evident from the graph that during the early stages of seedling growth the non-waxy and waxy endosperms decrease in weight at the same rate. During the latter

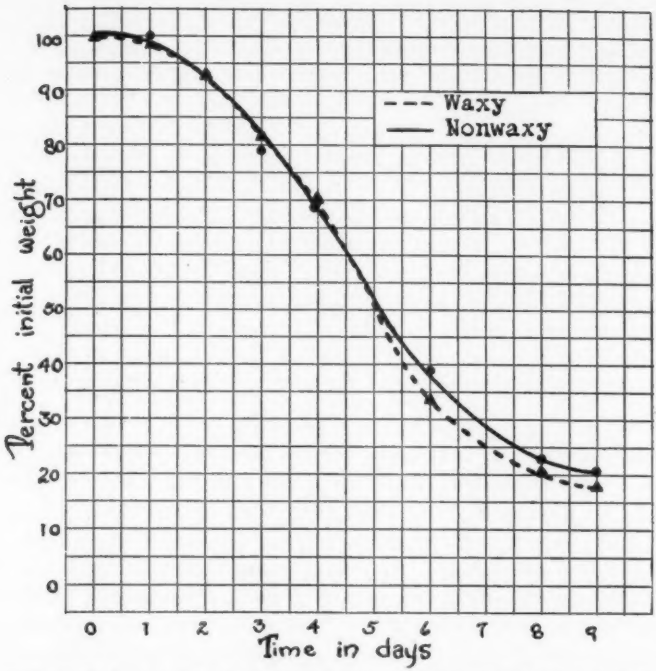

FIGURE 8.—Decrease in weight of the seed residues (endosperm and pericarp) during early growth of non-waxy and waxy maize plants in darkness. The curves represent the percentages of the inital amount of material remaining as the development of the seedlings progresses.

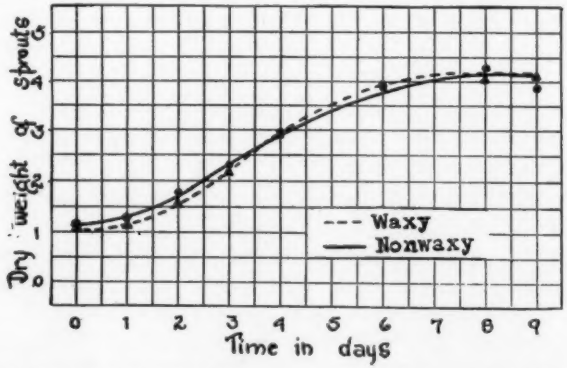

FIGURE 9.—Increase in dry weight of sprouts of non-waxy and waxy plants during the seedling stage. The plants were grown in darkness.

TABLE 7

Weight in grams of non-waxy and waxy sprouts during seedling growth. The values are adjusted to a basis of 50 individuals.

AGE OF SEEDLINGS IN DAYS	WEIGHT OF SPROUTS IN GRAMS	
	Non-waxy	Waxy
0	1.18	1.05
1	1.34	1.12
2	1.73	1.56
3	2.36	2.27
4	2.90	2.84
6	3.86	3.96
8	4.37	4.07
9	3.77	4.14

half of the cycle, however, it would appear that the waxy reserves decrease more rapidly, although the difference in mass of undigested material is never more than 5.1 percent and decreases to 3.5 percent on the ninth day. The results obtained in test 1, which are not reported in detail here on account of their incompleteness, agree in showing that from the fourth to the eighth day the waxy reserves are utilized somewhat more rapidly than those in the non-waxy seed. There may be, therefore, a significant though small difference in the rapidity with which the two kinds of starches are digested. In test 1, which was carried to the twelfth day, no further decrease in weight of the seed residues occurred after ten days. At this time the two types were alike in retaining about 15 percent of the original substance. It is probable, therefore, that both go to the same "end point."

During the seedling period the sprouts increase in weight nearly fourfold. As table 7 and figure 9 show, the difference between the non-waxy and waxy starches in this regard is probably not significant. The results obtained in test 1, as far as they go, also support this conclusion.

In view of the ease with which the waxy starch is hydrolyzed with amylase *in vitro*, the very close correspondence in rate of digestion of the non-waxy and waxy endosperms during seedling development calls for an explanation. Quantitative studies which we have made on the amylases produced during germination show that the amounts of this enzyme in the waxy seedlings is distinctly less than that formed by the non-waxy type. An interesting compensating feature is thus revealed, which, from the standpoint of our study on the nature of the waxy gene, may be highly significant. In waxy seed we have an easily digested reserve starch, but a relatively small production of amylase, whereas the starch of the non-

waxy variety shows a greater resistance to hydrolysis, but is accompanied by a larger amount of enzyme. It seems probable that these effects of the waxy factor bear a definite genetic relation to each other.

In the field, waxy plants attain the same development as their non-waxy sibs, which demonstrates that the waxy gene does not occasion the absence of any particular essential for the manufacture of protoplasm. The present results on weight of sprouts indicate that the time relations of the ultimate synthetic processes involved in growth are not appreciably altered. So far, therefore, as the general economy of the plant is concerned, the waxy gene does not cause any significant changes. If the waxy factor acted upon a variety of metabolic processes in the seedling, we might expect this to be reflected in an altered rate of growth. There is direct evidence that the gene modifies certain carbohydrate transformations. Where these may become limiting, as in pollen-tube growth (cf. BRINK 1925), we do find a change in the rate at which the individual develops. While it cannot be definitely concluded that there are no other effects, the evidence favors the view that the waxy gene is limited in its sphere of action to changes in carbohydrate metabolism.

DISCUSSION

The present investigations have been undertaken in the belief that we may gain some knowledge of the dynamic properties of genes by studying the physiological changes occasioned in an organism by the substitution of one allelomorph for another. The idea is nearly as old as genetics itself, although the work based upon it is not extensive. The early results on color inheritance in plants and animals aroused considerable speculation as to the chemical bases for the differences found. The Mendelian relations were often simple, and sufficient was known regarding the constitution of certain pigments to engender the hope that the hereditary phenomena might be interpreted in terms of chemical transformations. Workers of the English school have made some notable advances and have opened up a field, which, though beset with many difficulties, should yield further important results to the investigator with the requisite training in chemistry and genetics.

In Antirrhinum the formula *YYII* has been assigned to the ivory type and *YYii* to the yellow-flowered form, on the basis of their breeding behavior. Plants lacking *Y* are albinos. WHELDALE (1916) has shown that the pigments concerned here are two flavones, apigenin and luteolin. The yellow variety contains both these compounds, but the plants of the ivory class produce only apigenin. Albinos contain no flavone and this is

attributed to the absence of the *Y* factor. The *I* gene may be considered as responsible for the production of a substance which inhibits the formation of luteolin, or, since the latter compound is more highly oxidized than apigenin, *I* may be a reducing agent. Evidence is presented which indicates that each of these two pigments is built up of phloroglucin and an oxybenzoic acid. On treatment with caustic alkali apigenin forms P-oxybenzoic acid, whereas luteolin yields protocatechuic acid. The *I* gene may operate, therefore, to prevent the formation of this latter compound.

The existence of complementary factors for flower color has suggested the hypothesis that one of the germinal elements concerned gives rise to an oxidase which, acting on a chromogen formed by the other gene, produces pigment. This idea, however, has not been substantiated. WHELDALE (1916), working with Lathyrus and Matthiola, obtained qualitative evidence for flavones in both the white races which give color when crossed. Likewise oxidases proved to be present in both. WHELDALE considers, however, that the critical tests have not been made. A difference may yet exist in these white races in regard to a specific flavone concerned in the formation of the pigment. In regard to the oxidases, it is pointed out that these substances are probably not completely wanting in any plant, and if means were found of differentiating between various oxidases, a significant difference in this regard in the white strains might be revealed.

The survey made by KEEBLE and ARMSTRONG (1912) also showed oxidases in both the white-flowered sweet peas carrying complementary factors for color. Their results in general are strongly suggestive, nevertheless, of a close relation between these enzymes and pigment formation. They further found that the intact corollas of dominant-white *Primula sinensis* plants gave no reaction for oxidase. After treatment with dilute hydrocyanic acid, however, a positive test was obtained. These facts led the authors to conclude that "The results confirm the Mendelian hypothesis that dominant-white flowers owe their lack of pigment to the presence of an inhibitor of pigment formation."

Using the chemical evidence adduced by GORTNER (1911, 1912), ONSLOW (1915) and others, WRIGHT (1917) has elaborated an ingenious hypothesis to account for the color variations in mammals. The chemical evidence indicates that the pigments of the eye, fur and skin of mammals, known collectively as melanins, are formed from tyrosin or related products of protein metabolism through the agency of oxidizing enzymes. It seems probable that there are various chromogens, those used being dependent on the character of the enzymes present. The hereditary differences in color are due to differences in the kind or amount of enzyme

involved in the reactions attending pigment production. WRIGHT assumes two enzymes, I and II, color depending on the rate of production and potency of these.

"Enzyme I is essential to the production of any color, but by itself only produces yellow. Enzyme II is supplementary to enzyme I, producing no effect by itself. The compound enzyme, I-II, produces a darker kind of pigment than enzyme I alone, viz., sepia. Enzyme I-II is also more efficient than enzyme I in another way. It produces sepia pigment even when enzyme I is at too low a potency to produce any yellow by itself. Above the level at which enzyme I produces effects, the two enzymes, I and I-II, compete in the oxidation of chromogen. Chromogen which is oxidized by enzyme I to yellow pigment is incapable of further oxidation to black. In the mixture the presence of the relatively pale yellow colors serves mainly to dilute the color of the hair. This production of yellow reduces the amount of dark pigment and the apparent intensity of color, both by reducing the amount of enzyme I which can unite with II to form the enzyme for black production and also by using up chromogen which would otherwise become black."

WRIGHT interprets a very large array of breeding facts on this hypothesis, but points out that

"Any such assignment of physiological effects to factors is at present to be taken mainly as a means of visualizing their action in our ignorance of the real physiology."

While WRIGHT'S theory of gene action utilizes the available chemical facts, it rests mainly upon the hereditary relationships. It is clear that much further knowledge of the processes involved in the production of melanin pigments must be obtained before it can be measured against adequate chemical evidence.

In view of the direct effect of the waxy factor in the pollen of maize and the ease with which starch may be prepared from the grain and amylases extracted from the seedlings, it would seem that particularly favorable material is offered here for a study of the dynamics of a gene. The similarity of the reserve starch produced in waxy pollen and waxy endosperm makes it seem probable that the gene functions in the simple gametophytic structure as it does in the green plant in so far as carbohydrate changes are concerned, at least. There are three lines of evidence pointing to the conclusion that the main carbohydrate reserve in the pollen and endosperm of waxy maize is the same substance. In quantitative chemical analyses the chief storage product in each case is found to separate out in the starch fraction and microscopic examination shows starch grains in both tissues. The starches from these two sources, when prepared in pure form and dissolved in water, give the same color reaction with iodine, namely, Rood's Violet. And thirdly, they are alike in being more readily

hydrolyzed by amylase than non-waxy starch. The assumption is reasonably well founded, therefore, that the waxy gene governs the same series of reactions in the pollen as in the developing endosperm. The fact that these reactions occur in the simple binucleate gametophyte leads us to the view that they are primary effects of the waxy gene.

It has been our aim in the present investigations to determine so far as possible the difference in the nature of the reserve materials of the non-waxy and waxy races of maize as the first step in the analysis of the action of the waxy gene. Proximate chemical analysis shows at once that the view of WEATHERWAX (1922) that the reserve material of the waxy endosperm is erythrodextrin is not well founded. The term erythrodextrin, so far as we have been able to learn, was first used by BRÜCKE (1872) who applied it to the product obtained on roasting starch. Contending that this treatment gives a mixture of dextrins, LINTNER and DÜLL (1893), by repeated fractional precipitation with alcohol, claim to have prepared a pure erythrodextrin. This substance which gave a reddish-brown color with iodine, as its name suggests, was *readily soluble in water*. The reserve carbohydrate characteristic of waxy maize gives the red-brown color reaction by reflected light, but we find that it is not soluble in water or in 10 percent alcohol, the reagent employed in standard methods of analysis to extract dextrins from mixtures containing starch. BRÜCKE's (1872) preparation was probably what we know today as "commercial dextrin." This substance is also readily soluble in water. The waxy reserve, therefore, does not conform to either of the substances previously described as erythrodextrin. ARTHUR MEYER (1886), whom WEATHERWAX (1922) quotes as giving the only comprehensive account of this polysaccharide, does not use the term erythrodextrin. We must not be misled into thinking that the waxy reserve is necessarily a dextrin simply because it gives a red color with iodine; the animal physiologists have long recognized glycogen, which forms a reddish-brown compound with iodine, as having the properties of a starch. There seem to be no good reasons either chemical or historical, therefore, for calling the reserves of waxy endosperm erythrodextrin.

We prefer to look upon this material as a starch. If it must have a name we may with propriety follow MEYER (1886) in calling it "red-staining starch." This red-staining starch is characteristic of the endosperm of certain other cereals also. GRIS (1860) found it in *Oryza sativa* var. *glutinosa*, and MEYER (1886) established its presence in *Panicum miliaceum* var. *canditum glutinosum* and *Sorghum vulgare glutinosum*. It has also been found in another grass, *Coix lachryma-jobi* (LONGLEY 1924). From

the account given by TADOKORO and SATO (1923), it seems very probable that the endosperm reserves of glutinous rice are identical with those of waxy maize. Indeed, it appears likely that we have the same substance in all five species.

MEYER (1886) attempted an explanation of the occurrence of starch grains which stained red with iodine. He considered that the starch granules of glutinous rice and glutinous sorghum were composed of small amounts of starch, staining blue with iodine, some amylodextrin, giving a red color with iodine, and a third substance present in the largest amount which he called dextrin. This latter substance gave a brown color with iodine. According to the author the amylodextrin arises through the action of a weak ferment on the blue-staining starch deposited in the grain. TANAKA (1907) denies the presence of other compounds in the starch grains of glutinous rice and considers the red coloration with iodine a characteristic of the starch itself. TADOKORO and SATO (1923) have made a study of some colloidal properties of glutinous rice starch and conclude that it may differ from common starch only in the degree of polymerization.

In our own work we have examined the composition of the starches of non-waxy and waxy maize as revealed by centrifuging suspensions at high speeds, which treatment, as SHERMAN and BAKER (1916) found, serves to separate in a crude way the two principal constituents of the grain. Further, we have compared the rates at which these starches are broken down to compounds giving no color with iodine under the action of hydrolytic agents. Observations on the color reactions of the whole starches and their components after centrifugation have also been made. Although we cannot draw definite conclusions on the basis of the facts at hand, we may indicate some possible differences between the non-waxy and waxy starches. We shall consider the evidence regarding color with iodine first.

As we have already pointed out the α- and β-amyloses prepared from the two starches give the same color reactions as suspensions of the respective whole starches. The criticism might be made that the assumption of color by the relatively insoluble α-amyloses is due to their being contaminated with β-amylose. The fact, however, that the α-amylose fractions tenaciously retain their power of staining under the action of amylase and hydrochloric acid which rapidly hydrolyze the β-amyloses, leads us to think that the two components of each of the respective types of starch are really alike in this regard. Whatever this fundamental difference in organization is which causes waxy starch to form a red

compound with iodine, it characterizes both the fractions separated by centrifugation.

HARRISON (1911) has made a study of the color reactions between starch and iodine from the colloidal standpoint. He reaches the conclusion that the red and blue colors are due to differences in degree of dispersion of the iodine particles. Any agency, such as alcohol, potassium iodide or heat, which tends to bring the iodine into true solution, changes the color in the direction of blue to red. According to this view the ultimate particles of starch from waxy maize must be smaller than those of the non-waxy type, thus presenting a greater surface on which the iodine is distributed. This might indicate a lower degree of polymerization of the red starch, but it must be admitted that the present knowledge of the color relations of starch-iodine solutions is rather unsatisfactory.

The nature of the difference between non-waxy and waxy maize starch is a problem the ultimate solution of which must wait upon a knowledge of the constitution of the molecules involved. A few years ago this statement would have implied an abandonment of the problem, for it seemed then that the structure of the starch molecule was scarcely accessible to analysis. But in the preface to the fourth edition of his monograph on "The carbohydrates and the glucosides," ARMSTRONG (1924) remarks: "The outstanding advance, a very great one, recorded in this edition, is that relating to the complex carbohydrates, inulin, starch and cellulose." The work of IRVINE (1923) and his school, PRINGSHEIM (1923), LING and NANJI (1923) and others has given an insight into this puzzling question which presages for the near future a knowledge of the structure of the starch molecule far more precise than could have been hoped for by the organic chemist of a decade ago, who found the methods which had proved so fruitful in other directions of little avail with this complex polysaccharide. Naturally, in a field where the significant developments are so recent there are many points of controversy, but certain general deductions may be drawn, which, if they do not find direct application to our problem in its present stage, at least indicate the lines along which certain phases of it may eventually be solved.

As hydrolysis with a strong acid shows, starch is composed entirely of glucose residues. The problem the constitutional chemist has sought to answer is the number and arrangement of these residues in the basal unit, the molecule, and the manner in which these units are polymerized. IRVINE (1923) who has brought one particular method of inquiry, the methylation process, to a high degree of usefulness considers that the

basal unit in starch is a tri-amylose $(C_6 H_{10} O_5)_3$ and has deduced a structural formula in keeping with the results of the reactions studied.

LING and NANJI (1923), recognizing the heterogeneous nature of the starch granule, have first separated its two main constituents which they term polymerized amylose and amylopectin. In the starch of wheat, barley, rice and arrowroot they find that these substances are alike and are present in the ratio of 2 to 1. The starch grain may also contain varying amounts of another substance, probably a hemicellulose. LING and NANJI find that amylose and amylopectin yield different products on being hydrolyzed with appropriate enzymes. Thus, polymerized amylose when treated with precipitated barley diastase at 50° is hydrolyzed readily and quantitatively to maltose, whereas amylopectin under these conditions is scarcely attacked. The authors consider that the properties of amylose are best accommodated on the assumption that it is an α-hexa-amylose in which carbons 1 and 6 of the six glucose residues are united through oxygen atoms in the form of a ring compound. There are 6 α-linkings in this molecule the significance of which is that it may be hydrolyzed by maltase which attacks α-glucosides but not the stereoisomeric β-glucosides towards which emulsin has a specific action. The basal unit of amylopectin is considered to be a phosphoric ester of $\alpha\beta$ hexa-amylose. SAMEC (1914) previously demonstrated the presence of phosphorus as being in organic union with starch. LING and NANJI find that when amylopectin is treated at 70° with precipitated malt diastase previously heated to 70° a hexatriose ($C_{18} H_{32} O_{16}$) is formed. With emulsin this triose produces glucose and maltose, whereas glucose and iso-maltose are formed when it is hydrolyzed with yeast maltase or malt diastase. This indicates both α-linkings and β-linkings. Four of the former and two of the latter are postulated in the cyclic formula containing 6 glucose residues assigned to amylopectin.

Our experiments show that on heating 1 percent suspensions of non-waxy and waxy starch at 85° for 10 minutes and centrifuging at 2400 r.p.m. for 30 minutes, the starch separates into two layers, the upper one containing crude β-amylose and the lower one crude α-amylose. With this treatment the non-waxy starch yields about 8 percent of the former substance and the waxy starch about 60 percent. The yield of β-amylose from the non-waxy starch is about one-half that obtained by SHERMAN and BAKER (1916) on treating potato starch in this way.

LING and NANJI (1923), working with potato starch also, which, according to them, consists almost entirely of amylose and amylopectin, appear to have obtained much larger yields of amylose by their methods. The

different procedure we have followed makes it impossible, therefore, to judge with much assurance whether the large difference in proportions of α-amylose and β-amylose in non-waxy and waxy maize starch is indicative of a departure in one (or both) cases from the 2 : 1 ratio of amylose and amylopectin which LING and NANJI have found to hold with a number of other common starches. Nevertheless, the much larger amount of the more soluble constituent we have obtained from waxy starch cannot be without significance. It is indicative of a difference either in the above-mentioned ratio or in the proportion of hemicellulose in the starch grains.

We have shown that the starch from waxy maize is hydrolyzed about twice as quickly by 1 percent HCl as that from the non-waxy type and over sixty times as rapidly by salivary amylase. Furthermore, the precipitated amylases from both non-waxy and waxy seedlings digest β-amylose from waxy starch more rapidly than the corresponding fraction from non-waxy starch. These facts imply some profound differences either in the ultimate starch molecules themselves or in the manner in which these basal units are polymerized. In the absence of evidence concerning structure in these particular starches we cannot do more than indicate the various possibilities that may be involved. Knowledge of the constitution of the polysaccharides has now been advanced to the stage where this may be done in a rather definite way and we have the assurance that methods are available which put the solution of this phase of our problem within reach of the carbohydrate chemist. The starches from non-waxy and waxy maize probably differ in one or more of the following ways:

(1) In the structure of the basal unit in regard to
  (a) Number of hexose residues
  (b) Manner in which these hexose residues are linked, that is, the particular carbons involved and the stereoisomeric nature of the linkings.

(2) In the degree of polymerization with reference to
  (a) Number of molecules condensed together
  (b) Manner in which the molecules are condensed.

It is fully realized that the most important facts relating to the effect of the waxy gene on the nature of the starch reserves are still to be gained. We must look mainly to the constitutional chemist for these. Investigations on the structure of the starch molecule are now being pushed forward with vigor and the recent successes will undoubtedly encourage

further workers to enter this field. We may look forward with some confidence, then, to a much extended body of chemical data which may serve as a basis for interpreting the dynamic action of the waxy gene.

When tested under closely controlled conditions we find that waxy seedlings develop in the dark at nearly the same rate as those of the non-waxy type. During the latter part of the seedling stage the waxy reserves appear to be digested somewhat more rapidly as compared with the non-waxy type, but the sprouts do not increase in weight at a greater rate. This situation prevails in spite of the fact that the waxy starch is hydrolyzed by amylase *in vitro* much more readily than the other sort. The explanation of this probably lies, as we have already suggested, in the nature or amount of the amylases accompanying these reserves. A possibility especially worthy of consideration is that the type of reserve originally synthesized in the endosperm is determined by the amylase present. Substrate and enzyme might consequently bear such a relation to each other that the reverse process, namely, hydrolysis, would be effected at the same rate in the two types. It may be, therefore, that the waxy gene conditions, primarily, the kind or amount of amylase formed. Enzyme studies now in progress should throw light on this question.

SUMMARY

1. The direct effect of the waxy gene in maize on the type of reserve carbohydrate formed in the pollen suggests that the primary physiological action of this factor may be relatively accessible to analysis. The evidence presented indicates that the chief reserves in pollen and endosperm are probably identical, which implies that the same carbohydrate transformations occur in the two parts. The fact that this effect is encountered in the simple binucleate pollen is strongly suggestive of its being the immediate result of the activity of the waxy gene. The important practical advantage in the parallel action in the development of the seed is the abundance of material the latter affords for investigation.

2. Proximate chemical analyses show that the principal reserve carbohydrate in waxy pollen and endosperm is a starch. The starches from these two sources when prepared in relatively pure form and dissolved in water give the same color reaction with iodine, namely, Rood's Violet (RIDGWAY). They are alike moreover in being more readily hydrolyzed by amylase than non-waxy starch. It is considered probable, therefore, that the starches from pollen and endosperm are chemically identical.

3. Application of the name erythrodextrin to this substance is not in accord with previous usage of the term and is not justified by the chemical

and physical properties of the material. Solubility reactions in water and in 10 percent alcohol, separation of suspensions on centrifugation into two fractions and behavior on hydrolysis with amylase and HCl show clearly that the substance is a starch.

4. In form the starch grains from the endosperm of non-waxy and waxy seeds show only slight differences. In the pollen the granules are much smaller and the two types show a characteristic difference in shape. The two types of starch cannot be distinguished on the basis of their appearance with polarized light.

5. On centrifuging dilute suspensions prepared by heating at 85° for 10 minutes the starches are separated into two layers, the upper one being clear and more limpid, the lower, more opaque and viscous. Following MEYER'S terminology, we have designated these fractions crude β-amylose and crude α-amylose, respectively. The non-waxy starch yields about 8 percent of β-amylose by this method, whereas about 60 percent was found in waxy starch.

6. The α- and β-fractions from each of the respective starches give the same color reactions with iodine. The α- and β-amyloses from waxy starch are more readily hydrolyzed by amylase than the corresponding portions from non-waxy starch. It is concluded, therefore, that the non-waxy and waxy starches differ in regard to both these components.

7. On HARRISON'S hypothesis the violet color of waxy starch with iodine might be considered as indicating a lower degree of polymerization than prevails in the common types of starch.

8. Waxy starch is hydrolyzed by boiling 1 percent HCl about one and one-half times as rapidly as non-waxy starch.

9. With salivary amylase the difference in rate of hydrolysis is very much greater. On account of incomplete digestion the end points are more difficult to read, but it appears that the waxy starch is broken down by this enzyme at least sixty times as rapidly as common maize starch.

10. Amylases prepared from both non-waxy and waxy seedlings also hydrolyze the waxy starch at a greater rate.

11. Some recent important advances in the chemistry of starch are noted in the text, which indicate that a satisfactory solution of the structure of the starch molecule, which has so long defied analysis, is now within reach. While no information is available at present bearing directly upon the difference in the constitution of non-waxy and waxy starch, the possibilities in this regard may be formulated in a fairly definite way on the basis of the chemical knowledge now at hand. A study of waxy starch

along the lines developed by IRVINE and his associates and LING and NANJI might yield results of very great interest to the geneticist and the carbohydrate chemist also.

12. Waxy seedlings grown in the dark develop sprouts at the same rate as their non-waxy sibs. In the former the seed residue appears to decrease in weight somewhat more rapidly in the later stages, but the difference is much smaller than might be expected in view of the great ease with which waxy starch is hydrolyzed by amylase *in vitro*. The suggestion is offered that the type of reserve synthesized in the endosperm is controlled by the kind or amount of amylase present and that substrate and enzyme may bear such a relation to each other that the reverse process, hydrolysis, may proceed at the same rate in the two types of seedlings. On this hypothesis the initial effect of the waxy gene relates to the type or amount of amylase formed.

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